# INTERNATIONAL SCIENTIFIC CONFERENCE IN DENTISTRY 2023 NOVI SAD

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On behalf of the Organizing and Scientific Committee, we are pleased to welcome you all at the International Scientific Conference in Dentistry 2023 Novi Sad.

The Scientific and Organization Committee made an effort to make a conference that has scientific value and is also interesting for dental professionals, although it is held in hybrid form.

You will meet an exceptional program covering different topics, from basic research areas to areas within daily practice of Restorative Dentistry, Endodontics, Prosthodontics, Oral Surgery and Implantology, Periodontology, Pediatric and Preventive Dentistry.

President of the Organizing Committee Prof. dr Tatjana Puskar

President of the Scientific Committee Prof. dr Milica Jeremic Knezevic

# **TABLE OF CONTENTS**

FULL PAPERS LECTURERS
BIOACTIVE MATERIALS IN DENTAL PULP THERAPY 7
STATE OF THE ART AND DEVELOPMENT OF GOLD DENTAL ALLOYS FROM ZLATARNA CELJE
ROLE OF PHYSICAL THERAPY IN THE MANAGEMENT OF TRIGEMINAL NEURALGIA 19
SINGLE POSTERIOR IMPLANTS IMMEDIATE LOADING BY MEANS OF ONE ABUTMENT ONE TIME
DENTAL-PROSTHETIC RESTORATION IN SEMI-MOBILE AND IMMOBILE GERIATRIC PATIENTS IN CONDITIONS OF COVID-19 PANDEMIC
IN VITRO ACCURACY EVALUATION OF THREE DIFFERENT SCANNING PROTOCOLS33
CHAIRSIDE CAD/CAM RESTORATIONS: WHERE IS THE LIMIT?
DENTAL PLAQUE AND SALIVA MICROBIOME. DO WE KNOW ENOUGH ABOUT THEM?43
USE OF PIDAQ IN A SOCIODENTAL APPROACH FOR ESTIMATING ORTHODONTIC TREATMENT NEED
MANAGEMENT OF DEEP CARIES: CHALLENGES AND OPPORTUNITIES
DENTURE STOMATITIS AND OTHER CANDIDA–ASSOCIATED INFECTIONS IN THE ORAL CAVITY
EPIDEMIOLOGY OD HEPATIS C IN SPLIT-DALMATIA COUNTY
SOST AND DKK-1 AS BIOMARKERS FOR TREATMENT OUTCOMES OF CHRONIC STATIN THERAPY IN PERIODONTAL DISEASE
PERIODONTITIS AS A RISK FACTOR FOR THE DEVELOPMENT OF SYSTEMIC DISEASES
ADVANCES IN MODERN PREVENTIVE DENTISTRY
FROM PERIODONTAL HEALTH TO DISEASE: THE IMPACT OF SMOKING
APPLICATION OF CELL CULTURES IN DENTAL RESEARCH
VISTA - VESTIBULAR APPROACH IN PERIODONTAL AND PERI-IMPLANT SOFT TISSUE REGENERATION
DIODE LASERS IN DENTISTRY, DENTIN HYPERSENSITIVITY - THE BEST THERAPEUTIC APPROACH
PROBIOTIC SUPPLEMENTATION IN DENTAL CARIES PREVENTION
PREVENTION OF DENTAL CARIES IN CHILDREN
BIOMECHANICAL ASPECTS OF IMPLANT PROSTHODONTICS
REVIEW OF MODERN ROOT CANAL FILLING MATERIALS
SCREENING FOR LOSSES IN INTRINSIC CAPACITY OF OLDER PEOPLE IN THE DENTAL PRACTICE
COMPUTER ASSISTED OCCLUSAL ANALYSIS IN IMPLANT DENTISTRY
HOW TO PERFORM CORONECTOMY OF WISDOM TEETH? DIAGNOSTIC AND THERAPEUTIC TIPS AND TRICKS

CYTOKINES AS MOLECULAR PAIN BIOMARKERS IN PATIENTS WITH	
TEMPOROMANDIBULAR DISORDER	
CLINICAL ORIENTATION OF THE OCCLUSAL PLANE USING A MODIFICATIO BALKWILL ANGLE	

FULL PAPERS	127
ADVANCED TECHNIQUE FOR SMILE DESIGN IN PROSTHODONTICS AND AESTHE	TIC
DENTISTRY	128

ABSTRACTS
ODONTODISPLASYA - CASE REPORT
PAROTID GLAND SWELLING AFTER CHLORHEXIDINE MOUTHRINSE: CASE REPORT .134
THERAPY OF PSEUDOPROGENIA IN MIXED DENTITION
DISC DISLOCATIONS OF THE TEMPOROMANDIBULAR JOINT
THE CORRECT INDICATION – BASIC PRECONDITION IN DIASTEMA THERAPY137
DYNAMICS OF FLUORIDE ION RELEASE FROM TWO CONVENTIONAL GLAS-IONOMER CEMENTS: A COMPARATIVE STUDY
COMBINATION OF FULL- AND SPLIT-THICKNESS CORONALLY DISPLACED FLAP WITH CONNECTIVE TISSUE GRAFT IN THE GINGIVAL RECESSION TREATMENT
IMPROVEMENT OF THE HYBRID LAYER MORPHOLOGY BY DENTINAL COLLAGEN CROSS-LINKERS
COST ACTION 21122- PROMOTING GERIATRIC MEDICINE IN COUNTRIES WHERE IT IS STILL EMERGING. (PROGRAMMING)
THERAPY OF DISTAL BITE BY TWIN BLOCK APPLIANCE
APPLICATION OF SPHERICAL TIO2 NANOPARTICLES AS MODIFICATION OF GLASS- IONOMER CEMENTS
KNOWLEDGE OF DENTISTS AND PHYSICIANS ABOUT THE ASSOCIATION BETWEEN PERIODONTITIS AND SYSTEMIC DISEASES145
LIP PRINT PATTERN AS AN IDENTIFICATION TOOL146
BIOLOGICALLY BASED THERAPIES IN ENDODONTICS - LATEST STANDPOINTS FOR VITAL PULP THERAPY
ORAL MUCOSAL LESIONS IN CHILDREN
THERAPY PLANNING FOR SUCOMPLETLY EDENTULOUS PATIENTS
TESTS FOR INVESTIGATION OF CYTOTOXICITY IN DENTISTRY150
FREQUENCY OF RUBBER DAM USE AMONG DENTIST IN SERBIA AND REGION151

# FULL PAPERS LECTURERS

## **BIOACTIVE MATERIALS IN DENTAL PULP THERAPY**

#### Drobac M.<sup>1</sup>, Ramić B.<sup>1</sup>, Cvjetićanin M.<sup>1</sup>, Stojanac I.<sup>1</sup>, Petrović Lj.<sup>1</sup>

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#### Abstract:

Preserving the pulp in a healthy state with sustained vitality, preventing apical periodontitis, and developing minimally invasive biologically-based therapies are the key objectives of contemporary clinical endodontics. Bioactive endodontic cements (BECs), which mainly comprise calcium and silicate elements, have been shown in clinical trials to offer superior treatment outcomes. As a result, these materials are widely used in clinical practice and are the most extensively studied. In this paper, the chemical composition and the application of BECs for vital pulp therapy (VPT)—including indirect pulp capping, partial pulpotomy, full pulpotomy, and pulpectomy—are discussed.

Key words: bioactive materials, pulp capping, pulpotomy, vital pulp therapy

#### Introduction

Historically, absence of toxicity has been the primary biological condition for placing foreign materials into the human body. This criterion was modified in recent years to require that materials be "bioactive" [1], i.e., that they are capable of interaction with living cells and tissues as a means of inducing a specific response in them [2]. In dentistry, these outcomes may include increased adhesion to dentin or bone for stabilization and interfacial sealing [3], reducing interfacial degradation mechanisms that compromise the lifetime of polymer-based dental restorations [4], favorable interactions with the oral microbiota that may transform a system away from dysbiosis and tissue destruction [5], or stimulation of new mineral formation to replace hard tissues that have been lost due to damage or disease [6].

Mineral trioxide aggregate (MTA), developed in the 1990s, was introduced in dentistry for that purpose, and has shown excellent bioactive properties in practice [7]. As a bioactive endodontic cement (BEC), MTA mainly consists of calcium and silicate components [7] and is thus typically denoted as calcium silicate cement. Since then, a large number of calcium silicate cements have been developed, including BioAggregate, Biodentine, BioRoot RCS, calcium-enriched mixture cement, Endo-CPM, Endocem, EndoSequence, EndoBinder, EndoSeal MTA, iRoot, MicroMega MTA, MTA Bio, MTA Fillapex, MTA Plus, NeoMTA Plus, OrthoMTA, Quick-Set, RetroMTA, Tech Biosealer, and TheraCal LC.

They can be used in a variety of contexts involving loss of tooth structure, including dental caries treatment, restoration of dentition following trauma, and mitigation of tooth wear. However, if the pulpal tissue is endangered, a series of interventions must be undertaken with the goal of pulp preservation [8]. Such vital pulp treatment (VPT) procedures have traditionally included indirect or direct pulp capping, and partial or complete pulpotomy [9]. Previously, VPT was primarily applied to immature adult teeth, to preserve the radicular pulp and ensure completion of root formation (apexogenesis). However, in recent years, VPT applications have expanded to include root canal therapy (RCT) in mature teeth, including those previously considered to have irreversibly inflamed pulp [10]. Consequently, contemporary clinical endodontics is primarily concerned with promoting and preserving pulp health and vitality, preventing apical pathosis, and developing minimally invasive biologically based therapies [8].

Despite considerable advances in oral health, dental caries remains the most common cause of tooth tissue loss and consequent damage to the dental pulp. As deep and extremely deep dental caries can be dangerous, elimination of all infected tissues is essential, which typically involves complete removal of both demineralized enamel and infected dentin [10]. Deep dental caries refers to a carious process that is very deep and close to the pulp tissue, but with a zone of hard or firm dentine between the

caries and the pulp. Nonetheless, during operative treatment, there is a risk of pulp exposure [8]. On the other hand, in extremely deep dental caries, the entire dentine thickness is affected by the disease, due to which pulp exposure cannot be avoided during caries removal [8]. Clinically, three types of dentin are distinguished in carious lesions—soft, firm, and hard dentin. Soft dentin can be removed by using hand instruments, whilst firm dentin is resistant to hand excavation methods, and hard dentin is sound and resistant to probing and scratching [8]. Depending on the clinical case, either selective or non-selective caries removal should be adopted, as the former method can target either soft or firm dentine, while the latter results in the complete removal of both soft and firm carious dentine.

#### Indirect pulp capping (IPC)

Indirect pulp capping can be adopted for both primary and permanent teeth [11]. For primary teeth, the Hall technique (sealing all carious dentine beneath a crown) has been found in extant research to be more cost-effective in treating carious primary molars compared to conventional treatment, which involves removing caries in its entirety followed by cavity restoration [12]. Clinical trials conducted by Mathur et al. and Menon et al. in 2016 provide further clinical (no pain, absence of sinus tract) and radiographic (no signs of external or internal resorption, presence of calcified bridge) evidence supporting successful outcomes following the use of MTA and calcium enriched cement (TheraCal LC) for indirect pulp capping in primary teeth [13,14].

IPC based on calcium-enriched mixture cement (CEM) [15] and MTA, medical Portland cement, Biodentine, and TheraCal LC as indirect pulp capping agents [14] has led to successful outcomes when applied to permanent teeth. However, patients provided IPC treatment need to be followed-up regularly, given that significantly higher success rates for bioactive endodontic cement (ProRoot MTA) compared to calcium-enriched mixture cement (Dycal) as an indirect pulp-capping material was demonstrated by Leye Benoist and colleagues three months upon treatment completion, but no significant difference was found between the two materials at 6-month follow-up in terms of calcified bridge thickness [16]. As this is one of a very few clinical and radiographic investigations on BECs as indirect pulp-capping materials, further research is needed to establish the most optimal treatment protocol, which should be based on the level of caries activity (the color or texture of the carious dentine) and depth [17].

#### Direct pulp capping (DPC)

Direct pulp capping (DPC) involves covering healthy or reversibly inflamed pulps that have been exposed mechanically or during caries removal. According to the current guidelines, only mechanically exposed healthy pulp should be considered for DPC [18]. However, understanding of pulpal repair mechanisms has highlighted the need for a low-grade inflammatory process to stimulate the regenerative response [19]. According to the pertinent findings, when the irritant is removed, the pulp has the capacity and potential to promote up-regulation of odontoblastic activity (reactionary tertiary dentinogenesis) or the recruitment of progenitor cells, which can cytodifferentiate into odontoblast-like cells (reparative tertiary dentinogenesis). After pulp exposure, odontoblast-like cells have the capacity to form reparative dentine, whereby the newly-formed hard tissue (mineralized bridge) should replace the lost dentine if this process is successful. Although calcium hydroxide has been the 'gold standard' capping material for years, recent reviews indicate that superior outcomes can be attained using bioactive endodontic cements [20]. The biological properties of these materials have been demonstrated in both in vitro (Careddu & Duncan, 2018) and in vivo (Parirokh et al., 2018) studies, as well as in clinical trials involving comparisons with other materials [21,22]. Available evidence further indicates that the hard tissue bridges formed against BEC exhibit greater histological quality relative to those induced by calcium hydroxide [23].

#### Partial pulpotomy

Partial pulpotomy involves removal of a small portion of coronal pulp tissue after exposure, followed by application of a biomaterial directly onto the remaining pulp tissue prior to the placement of a permanent restoration.

In this context, bioactive endodontic cements—such as calcium silicate cements and mineral trioxide aggregate (MTA)—have demonstrated superior histological [23] and clinical outcomes compared with

calcium hydroxide in the treatment of exposed pulp [20,22]. For this purpose, resin-based composites and dentine-bonding agents have been investigated, and the findings have shown that they should not be used in practice due to cytotoxicity [24], absence of dentine formation over the wound site, and poor clinical outcome [25].

#### Full pulpotomy

Full pulpotomy implies complete removal of the coronal pulp and application of a biomaterial directly onto the pulp tissue at the level of the root canal orifice(s), prior to the placement of a permanent restoration. According to Parirokh and colleagues, using BEC as a pulpotomy agent is associated with higher success rates compared to other examined pulpotomy agents [17]. When BEC is used for VPT procedures (DPC and partial pulpotomy) in permanent teeth with asymptomatic irreversible pulpitis, success rates at 1- to 2-year follow-up range from 85% to 100% [26]. On the other hand, in clinical trials, calcium hydroxide, glass ionomer cements (GICs), and resin-based materials demonstrated more variable, as well as considerably lower success rates, ranging from 43% to 92% [27].

#### Pulpectomy

Pulpectomy refers to the total removal of pulp from the root canal system followed by root canal treatment, ending with permanent restoration placement. In teeth with both closed and open apices, bioactive endodontic cements could be used as either root filling materials or root canal sealers in combination with gutta-percha for filling root canals [7]. Published research findings indicate that the clinical and radiographic outcomes of such interventions are comparable to those relying on gutta-percha and root canal sealers [28,29].

#### Conclusion

Silicate materials possess favorable physicochemical characteristics that include high alkalinity, intratubular mineralization, inhibition of biofilm formation, reduction of robust pro-inflammatory mediators, and less severe postoperative pain following dental pulp treatment. Immunomodulatory effects of the new generation of biomaterials provide further and much needed benefit to their biocompatible, osteogenic, and bioactive properties. In particular, formation of mineralized barriers using BECs results in superior restoration quality compared to calcium hydroxide-based materials. The newer BEC generations—including modified compositions that reduce tooth discoloration—also reduce setting time. The biomaterial choice must therefore be guided by the available scientific evidence as well as patient-centered outcomes, reliable mineralized tissue formation, and continued pulp vitality.

Further understanding of the processes of inflammation, repair, and material interaction is important for a better-informed application of these techniques and the development of novel diagnostic and therapeutic solutions.

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## STATE OF THE ART AND DEVELOPMENT OF GOLD DENTAL ALLOYS FROM ZLATARNA CELJE

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#### Abstract

The paper presents a historical overview of the production of gold dental alloys, the current development of this field, and the basic and functional properties of dental alloys. Gold dental alloys produced by Zlatarna Celje d.o.o. belong to the group of certified medical devices of class IIa in accordance with Regulation (EU) 2017/745 and represent a medium level of risk. Among these, gold dental alloys have a special status for porcelain crowns and bridges, where it is necessary to ensure the necessary requirements of such dental-prosthetic systems with the chemical composition, properties and production.

Key words: state of the art, development, Au dental alloys, properties, application

#### Introduction

Zlatarna Celje d.o.o. from Slovenia is constantly working on development and improvement of high gold content dental alloys with selected combination of the following alloying elements: Pd, Pt, Ag, Zn and: Ir, In, Rh, Nb, in traces. The basis for such an approach is the search for the most appropriate chemical composition, where the gold content is higher than 76 wt.%, and consistent manufacturing technology [1,2]. The demands, which must be met, with improvement of the gold dental alloys are linked with fulfilling the necessary requirements that makes alloy suitable to use for metal-porcelain ceramic restorations. Due to the stress generated by masticatory forces, dental alloys must have corresponding mechanical properties (yield strength, tensile and bending strength, elongation, etc.), hardness and required coefficient of thermal expansion (CTE); they must also be highly biocompatible [3,4]. Based on the written requirements, the production of the entire assortment of gold dental alloys must be carried out in accordance with Regulation (EU) 2017/745, in which such dental alloys are classified as a medical device of class II a - with a medium level of risk for the organism. Compliance of Zlatarna Celje d.o.o. gold dental alloys with the aforementioned regulation is guaranteed by a CE mark declaration. All gold dental alloys are registered in the Register of Medical Devices at the Public Agency of the Republic of Slovenia for Medicines and Medical Devices (JAZMP). Within the framework of the development and monitoring of the quality of Au dental alloys, Zlatarna Celje pays special attention to verifying the adequacy of not only their basic but also other functional properties, which include magnetic properties.

The paper will present the starting points that are strictly followed by Zlatarna Celje d.o.o. with controlled production, certified products by authorized domestic and foreign institutions, and continuous investments in development and research, to ensure optimal conditions for the production, control of dental alloy properties and the launch of health-friendly gold dental alloys.

#### State of the Art

It is known that dental prostheses and prostheses made of metal have been made and used for centuries. Thus, in the 19th century, dental prosthetic substitutes or dental cavities were made from substitutes by pressing aluminum, amalgam, gold, lead, platinum and silver. In 1907, precision casting was introduced [5,6], which enabled more sophisticated manufacturing of dental crowns and bridges. With the use of electric furnaces and casting equipment, the development of casting gold and other dental alloys advanced rapidly. Nowadays, modern digital techniques are used in the production of

dental prostheses, but despite this, many dental prosthetic prostheses are still made by casting. On the other hand, a review of the literature shows that different dental alloys have developed over the decades depending on their chemical composition, and the driving force behind their development has been technological changes in dental prostheses, progress in metallurgy and manufacturing techniques, the introduction of knowledge of functional properties and changes in price trends of precious metals.

The next major advance in the science of dental alloys and their treatment is the use of appropriate standards. In 1928, the American Dental Association (ADA) established the Dental Research Fellowship [7]. The work at ADA involves and is divided into several categories, including the measurement of clinically relevant physical and chemical properties of dental alloys and the development of new dental materials, instruments and test methods. In 1966, the ADA Council on Dental Materials and Devices was established and in the following period assumed responsibility for standards development and began product certification. The established standards define the requirements for the physical and chemical properties of dental materials, which ensure safe use if the dental technician and dentist handle and use the dental material correctly.

#### **Basic** properties

The use of all dental materials, especially metallic ones, including dental alloys, is based on their atomic structure. The common physical and chemical characteristics of dental alloys derive from their structure or microstructure, which is directly related to the properties of the dental alloy. Knowledge of these processes is thus related to physical chemistry, solid state physics, metallurgy and characterization. Since fundamental principles of physical science, engineering, and microstructure affect the properties of all materials, it is necessary to first study the microstructural characteristics before identifying the macrostructural characteristics.

Gold is the oldest restorative material for teeth. Early dental applications were based on aesthetics rather than chewing ability. The use of gold in dentistry is still important today [8]. In conservative and restorative dentistry and in orthodontic practice, gold is less often used as a pure metal, gold dental alloys dominating, which are alloyed with various precious metals or other metals necessary to achieve the required final properties.

An interesting new variant of gold dental alloys is represented by alloys with an extremely high gold content (> 85 wt.% Au) with the addition of platinum (around 10 wt.%) and with small additions of indium (0.2 wt.%) and zinc (0.1 wt.%) (so called Bioker) – [9] – see Figure 1. These high-gold dental alloys are characterized by lower strength, so they are not suitable for long-span bridges. It is known that the high gold content means that such a dental alloy, when combined with porcelain, has a warm colour similar to dentin. Due to this feature, gingival disease, i.e., is completely eliminated with these dental structures -black line, which is associated with traditional fixed restorations made of porcelain and base alloys. Dental alloys with a high gold content (76.6 wt.% Au) with additions of Pd, Pt, Ag, Sn, Ir and/or In have been used for many years for these reconstructive purposes [10]. This dental alloy (so called Aurokeram 2) has a fine-grained microstructure, sufficient strength for crowns and bridges.



Figure 1: BIOKER dental alloy for porcelain technique

#### Biocompatibility and corrosion properties

Clinical studies of dental alloys date back to the early 60s and 70s of the last century, and at that time depended on the entry of these alloys into various markets and the requirements that regulated their sale. For dental alloys, based on long-term use, we can prove their clinical relevance with a preclinical assessment of biocompatibility, which includes corrosion and cytotoxic tests. These tests determine the deterioration of the mechanical properties and effectiveness of the dental structure in the oral environment, as well as the release of elements from the dental alloy that can directly irritate the oral tissue next to the dental prosthesis or be released into the body. Potential systemic and local toxicity, allergy and carcinogenicity are the result of elements from the dental alloy being released into the oral cavity during the process of corrosion or use in the mouth. There is otherwise extremely little evidence that dental alloys cause systemic toxicity.

The basic condition and most important property for the use of a dental alloy is its corrosion resistance in the oral environment. The dental alloy must not affect electrochemical corrosion in the presence of other metals (gold, amalgams, etc.), or it must have a protective layer on the surface that reduces electrochemical potential differences and the release of ions from its surface. The formation of this layer is called passivation - otherwise known as the formation of a passivation layer that prevents the corrosion process from continuing. There is a well-known requirement that this passivation (protective) layer must not significantly change the colour of the dental alloy. On the other hand, there may be a situation where electrochemical corrosion products bind to proteins and form albuminates with heavy metals - happens and cause an allergic hypersensitivity reaction, which is reflected as local irritation of soft tissue: gingiva, cheeks, tongue. This is identified as burning tongue, dry mouth, metallic taste, canker sores, etc. in the mouth of a patient who has a dental prosthesis. For this reason, it is important to study the release of ions of individual elements from the surface of dental alloys in the area of the mouth and saliva. Investigations of various dental alloys revealed that in vitro cell assays identified gold (Au), palladium (Pd), platinum (Pt) and indium (In) as having no cytotoxic effect, while chromium (Cr), copper (Cu) and silver (Ag) toxic, respectively nickel (Ni), zinc (Zn) and cobalt (Co) very toxic [4]. Thus, an increased release of metal ions was detected in non-precious dental alloys (Co-Cr or Ni-dental alloys) and thus a greater possibility of the occurrence of allergies and inflammations in these alloys. In the case of noble dental alloys, it is known that the release of metal ions is greatly reduced, which is typical for these dental alloys. In the case of precious dental alloys, only zinc (Zn) is usually released in smaller quantities, which does not cause tissue inflammation around the dental prosthesis. Thus, the tissue around the tooth replacement made of a noble dental alloy has a significantly healthier appearance for many years after reconstruction, in some cases even up to several decades. Mechanisms of ion release depending on the chemical composition of dental alloys are shown in Figure 2, where it can be seen that high gold dental alloy has practically no released ions.

In modern medicine, an allergy to nickel is well known, which occurs when the skin is exposed to this metal. Approximately 20% of people have an allergic reaction (inflammation) to nickel, but it occurs more often in women, probably due to greater exposure to this metal due to wearing non-precious jewelry (such as earrings or piercings). Statistics for the monitoring of such allergies show that the incidence of nickel allergy in the population is increasing. For this reason, according to legislation, the proportion of nickel in products that come into contact with the skin or tissues has been decreasing in recent years. Therefore, palladium (instead of nickel) is used for the production of noble dental alloys. Palladium mixes easily with gold to make dental alloys and prevents silver from corroding in the oral cavity. Also, due to its similar properties to platinum, it is often used as a replacement element for platinum in dental alloys. Until recently, the purchase price of palladium was low, but recently, due to its use in the needs of electronic components, the consumption of palladium has increased greatly, resulting in an enormous increase in its price.

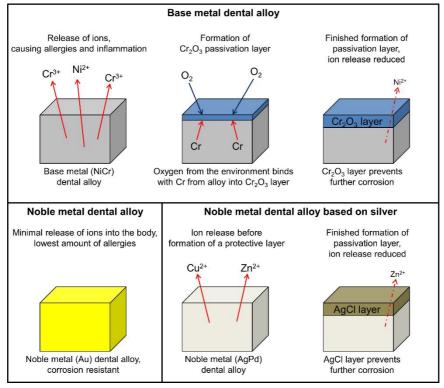


Figure 2: Schematic representation of ion release in the oral cavity depending on the chemical composition of dental alloys

The requirements within the standards are constantly evolving, with existing specifications being regularly revised, requiring changes in the formulation of dental products. This further represents that new knowledge about the behavior of dental materials in the oral cavity is taken into account. It should be borne in mind that no restorative dental material or dental device is completely safe. Safety is relative, and the choice and use of dental materials or appliances is based on the assumption that the benefits of such use outweigh the known biological risks. However, there is always uncertainty about the likelihood that a patient will experience side effects from dental treatment. There are two main biological effects: an allergic and a toxic reaction. This means that, in addition to functional efficiency and aesthetics, biocompatibility is an important requirement for dental materials in tooth restoration. For several years now, research has been trying to find a connection between the corrosion behavior and the cytotoxic effects of dental alloys. However, it is reasonable to state that the relationships between corrosion, released corrosion products and cytotoxic substances have not yet been clearly established. In order to predict the biocompatible properties of dental alloys, it is necessary to have information about their behavior in the potential dissolution of the surface, and we managed to investigate this in our own research [11]. For this reason, we tested the entire group of gold dental alloys from Zlatarna Celje. In the research work, we compared platinum and nine commercial dental alloys with a noble metal content of 27 wt. % to 97.6 wt. % according to the procedure described in the ISO 10271:2009 standard at pH = 7.2. The obtained values of the characteristic parameters of dental alloys with different content of noble metals, where only Au, Pt and Pd were considered noble, were compared with the classification system of the American Dental Association (ADA). Based on the obtained results, we classified the considered dental alloys of noble metals into three groups.

- In the first stability group, the so-called most stable dental alloys can be classified the highnoble dental alloys with the chemical composition of at least 60 wt.% of noble metals, and with gold content at least 40 wt.%;

- In the second stability group, the so-called stable alloys can be classified the noble dental alloys with chemical composition with at least 25 wt.% to 60 wt.% of noble metals,

- In a third group, the so-called unstable alloys, can be classified predominantly base dental alloys, with chemical composition less than 25 wt.% noble metals.

#### Magnetic properties

In the periodic table, only certain elements have magnetic properties [12]. These elements are iron, nickel, cobalt. In nature and in our immediate environment, these elements are most often found in mixtures or alloys. Magnetic properties are related to magnetism, which is a physical phenomenon where certain substances exert a repulsive or attractive force on other substances. This causes the movement of charged particles, which causes the formation of a magnetic field (Figure 3). Magnetism is present in all substances, but in some substances it is so weak that we cannot detect it without special preparations. The resulting magnetic field is a vector field around permanent magnets or conductors through which an electric current flows. In this field we perceive the magnetic force. It is illustrated by lines of force - lines that originate from the north pole of a magnet and converge to its south pole. The tangent to the line at each point is the magnetic field density.

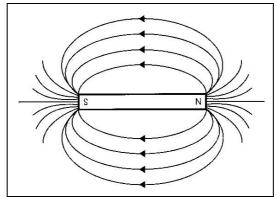


Figure 3: Magnetic field

We know several types of magnetism.

Diamagnetism results from the movement of electrons in atoms. It is a phenomenon when the density of the magnetic field in a substance placed in a magnetic field is slightly lower than the density of the magnetic field outside this substance (this means that the substance pushes the magnetic field out of itself). It is present in all substances, except for paramagnetic and ferromagnetic substances, where other, stronger phenomena dominate. Perfect diamagnetics are superconductors that completely push out the magnetic field.

Paramagnetism is a phenomenon when the magnetic field density in a substance that has been placed in a magnetic field is slightly higher than the magnetic field density outside this substance (it is assumed that the relative permeability  $\mu r > 1$ ). Such substances are called paramagnetic and usually  $\mu r$ is only slightly greater than 1. Paramagnetism originates from the electron's own magnetic moment, which is not a consequence of its motion. In an external magnetic field, the magnetic moments in a paramagnetic substance behave like magnetic fields and are partially arranged so that, on average, a few more of them point in the direction of the external magnetic field.

Ferromagnetism is the phenomenon that the density of the magnetic field in a substance placed in a magnetic field is much greater than the density of the magnetic field outside that substance. Ferromagnetism is the result of the fact that magnetic dipoles in ferromagnetic substances within macroscopic regions spontaneously arrange themselves and are arranged even outside the magnetic field. In an external magnetic field, the magnetic moments of these domains are directed in the direction of the external magnetic field. Unlike paramagnetism, which is present in both solids, liquids and gases, ferromagnetism is only present in rare solids. Among them are iron, cobalt, nickel and some alloys.

As part of determining the type of magnetism in the best-selling dental alloys, Zlatarna Celje d.o.o. we used: Bioker (Au 85,8 wt.%, Pt 11,7 wt.%, Zn 1,5 wt.%, In, Ir < 1 wt.%) and Aurokeram 2 (Au 76.6 wt.%, Pd 8,9 wt.%, Pt 9,0 wt.%, Ag 2,0 wt.%, Sn 1,0 wt.%, In 1,9 wt.%). The measurement was carried out on a SQUID magnetometer at the Institute of Physics, Belgrade, Serbia. The dental alloy plate had dimensions of 2 mm × 2 mm with a thickness of 1 mm and were polished and cleaned in an ultrasonic cleaner before measurement. Figure 4 shows the results of ferromagnetism measurements of these dental alloys [13].

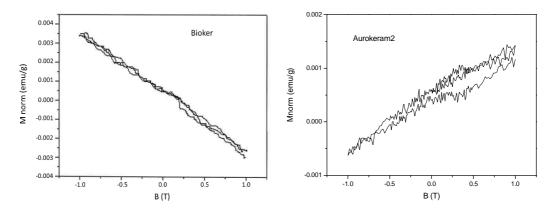


Figure 4: Magnetization curve [B/H] for Bioker and Aurokeram 2 dental alloy

Based on the measured magnetization curve [B/H] and their obtained characteristic shape (see pictures above), dental alloy Bioker belongs to diamagnetic materials, where the magnetic flux density inside is lower than the magnetic flux density outside. Dental alloy Aurokeram 2 belongs to paramagnetic materials, where the magnetic field density in the substance placed in the magnetic field is slightly higher than the magnetic field density outside this substance (relative permeability  $\mu r > 1$  is considered). The examined dental alloys are not ferromagnetic. Based on this, Zlatarna Celje can issues a certificate of non-ferromagnetism of the following dental alloys.

Magnetic resonance (abbreviated as MR) is a non-invasive medical diagnostic method in which the internal structure of the human body is imaged with the help of a magnetic field, radio waves and computer technology. The imaging shows in great detail the structures inside the skull, spine, limbs and especially the soft tissues of the head and body. The results make it possible to detect and precisely locate any injuries, neoplasms or other abnormalities, thereby helping to diagnose the patient's health problems and, consequently, to the success of the treatment. In the instructions for the preparation and course of the MR examination, it is written that before entering the examination room it is necessary to remove all metal objects from the body  $\rightarrow$  such as e.g. when examining the head or neck, where patients may also have a dental prosthesis installed. The problem arises when the dental prosthesis is fixed and cannot be removed. A key role in this research is represented by ferromagnetism, which is a phenomenon when the magnetic field density in a substance placed in a magnetic field is much greater than the magnetic field density outside this substance. This phenomenon is characteristic of Fe, Co, Ni and some other alloys, while the phenomenon of ferromagnetism is completely absent in precious metals and alloys. All precious dental alloys of Zlatarna Celje d.o.o. have the property of being non-ferromagnetic and do not pose a risk in MR examinations. This means that the precious dental alloys of Zlatarna Celje do not prevent the performance of MR examinations, even if the examinee (patient) has them embedded in the oral cavity. For the needs of MR examinations, we can issue an official statement on the nonferromagnetism of dental alloys to patients who have dental alloy prosthetics implanted in their mouths from Zlatarna Celje, which they submit to the questionnaire before the MR examination.

#### Conclusion

The state of development of dental alloys in the future must be aimed at achieving the following requirement with the goal of minimizing biological risks, dentists should recommend to patients dental alloys with the lowest possible ion release (absence of corrosion) and absence of ferromagnetism. This goal can be achieved by using highly noble or noble dental alloys with stable microstructure. However, there are exceptions here as well, so care must be taken to choose the correct dental alloy for each individual patient. For this reason, there are several dental alloys on the market that are suitable for individual patients with different allergies depending on dietary habits or other specific conditions in the oral cavity and tissues. In this case, it is necessary to follow the Zlatarna Celje recommendations for the dental alloys in order to prevent any uncertainty and thereby achieve a high quality dental prosthetic construction.

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# ROLE OF PHYSICAL THERAPY IN THE MANAGEMENT OF TRIGEMINAL NEURALGIA

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#### Abstract

Trigeminal neuralgia is an episodic chronic pain condition that has an incidence of approximately 4.5 per 100,000 and is typically seen in middle-aged or elderly patients, mostly women. An ideal treatment is the one that causes no morbidity and preserves the normal sensation of the face. Transcutaneous electric stimulation of trigeminal nerve is an emerging and promising option for treatment of trigeminal neuralgia. A low-level laser produces photo-biochemical reactions that can result in pain relief in patients with trigeminal neuralgia. Research on animal models has shown that therapeutic ultrasound produces a long-lasting antinociceptive, hypoalgesic effect on trigeminal neuralgia. Physical modalities are safe, non-invasive, least expensive and may prove to be an excellent option.

*Key words: Trigeminal neuralgia, Low level laser, Therapeutic ultrasound, Transcutaneous electric stimulation.* 

#### Introduction

Trigeminal neuralgia (TN) is an episodic chronic pain condition that has an incidence of approximately 4.5 per 100,000 people. TN typically affects middle-aged or elderly patients, mostly women with a ratio of 1.5–2:1, respectively [1]. Pain in TN is often extreme, sudden and unexpected, as well as short-lasting and patients describe that it has is stabbing, electrical shock-like, or shooting quality. Localization of pain is usually unilateral, with the distribution of one or more branches of the trigeminal nerve. Right-sided symptoms are more predominant, while pain occurs in paroxysms which last from a few seconds to a few minutes [2]. This condition can be triggered by normal activities, such as talking, chewing, or swallowing. The frequency of paroxysms often differs among patients from a few to a hundred daily. TN often disrupts activities of daily living and impacts life quality in affected individuals. It can be severely disabling and cause considerable mental distress, depression, and anxiety particularly among the elderly [3].

Pathogenesis and etiology of TN are not jet completely understood, and several theories are proposed. Peripheral theories are based on peripheral axon and myelin damage which cause erratic hyperactive functioning of the nerve. Central theories emphasize the importance of dysfunctions of brain stem, basal ganglion, and cortical pain modulatory mechanisms [4]. Certain conditions by causing nerve compression can trigger TN. These conditions include intracranial and extra cranial tumors or vascular anomaly as well as pressure of dilated and tortuous arteries in vicinity of trigeminal ganglion. Periodontal diseases, traumatogenic occlusion, nerve degeneration of deciduous teeth, circulatory insufficiency of trigeminal ganglion, multiple sclerosis, infectious agents, or age-related brain sagging syndrome are also considered as causative factors [5]. The diagnosis of TN is primarily based on patient history, as there are no definitive laboratory or diagnostic tests. Although patients do not have any abnormal neurological findings and display normal blink reflex, quantitative sensory testing detects subtle sensory abnormalities which may not be detected in routine clinical examination [5, 6] Magnetic resonance imaging (MRI) imaging is used to detect changes in trigeminal root, any neurovascular conflict and to rule out secondary pathology.

#### Treatment

Many treatment modalities have been described for trigeminal neuralgia. First line of drug therapy is reserved for sodium channel blockers, carbamazepine, or oxcarbazepine. However, side effects are quite frequent including somnolence, drowsiness, dizziness, rash, and tremor. High dosages are often necessary for adequate pain relief, so many patients have severe side effects. If carbamazepine or oxcarbazepine cannot reach full dosage because of side effects, combination treatment with lamotrigine, baclofen, pregabalin or gabapentin should be considered. Each of the above-mentioned drugs may also have efficacy when used as monotherapy, although the available evidence is very weak [6].

Subdermal therapies include application of Botulinum toxin type-A and subcutaneous alcohol blockage. Positive clinical response has been reported in over 70% of patients who underwent subdermal application of Botulinum toxin with no major side effects. Subcutaneous alcohol blockage to the affected trigeminal branches, provided excellent pain relief, lasting 2-30 months [7]. In medically refractory patients, with a neurovascular conflict, microvascular decompression is the first-choice treatment. Surgical microvascular decompression can control pain in more than 90% patients in short term period, however this rate decreases in long-term period with an annual recurrence of about 3,5%. Gamma knife radiosurgery is a non-invasive neurosurgical procedure that targets the trigeminal root, which is lesioned by convergent beams of radiation. The stereotactic irradiation of the nerve causes an electric block of the ephaptic transmission maintaining a normal axonal sensory conduction. The initial clinical efficacy of gamma knife radiosurgery starts from 2 weeks to 2.7 months after treatment, with a total pain relief after a median time of 5 months after. Pain relief is maintained in 46-65% of patients at 5-years follow up time. Second choice neurosurgical treatments are lesioning peripheral procedures targeting the trigeminal ganglion chemically by glycerol blockade, mechanically by balloon compression, or thermically by radiofrequency thermocoagulation [6, 7].

#### Physical therapy in the management of trigeminal neuralgia

Transcutaneous electric stimulation (TENS) of trigeminal nerve is an emerging and promising option for treatment of trigeminal neuralgia. The analgesic mechanism of TENS includes various factors: gate control theory, physiological block, and activation of endogenous pain inhibitory systems. Gate control theory is based on the fact that substantia gelatinosa in the spinal cord acts as a pain gate control system. Activation of large myelinating fibers subserving touch, pressure, vibration facilitates presynaptic inhibition of substantia gelatinosa cells in the dorsal horn, thus, reducing pain transmission [8]. Furthermore, as the frequency of stimulation increases, conduction decreases resulting in physiological block. Several studies demonstrated that TENS could also activate endogenous opioid systems operating both at spinal and supraspinal levels [9, 10]. Use of TENS for pain control in TN has several advantages: it causes prompt inhibition of pain at peak progression, it exibits no adverse effects and is non-invasive. Short term treatment for 20-40 days can result in pain reduction as compared with long term medicinal treatment. The application of TENS is simple, and it can be used at home with portable machine. TENS as a pain relief is equally effective in post neurectomy and after lesioning peripheral procedures [11].

A low-level laser produces photo-biochemical reactions that can result in pain relief in patients with trigeminal neuralgia. This method employs a mono wavelength of light, that can reach the neural tissue, and promote neurogenesis, the generation of new pathways, and neural cells [12, 13]. Low level lasers decrease acute and chronic pain using different mechanisms such as decreasing the level of histamine, acetyl choline, serotonin, bradykinin, and prostaglandin E2. They also increase levels of acetyl choline esterase, promote lymphatic drainage, aerobic metabolism, and pain threshold. Production of beta-endorphins and enkephalins is stimulated, balancing the activity of adrenaline-noradrenaline, and decreasing the production of P substance in the posterior spinal horn [5, 6, 7]. Low-level laser therapy (LLLT) improves the capacity for myelin synthesis and increases nerve function in injured nerves, according to clinical trials. Moreover, it promotes axonal growth in wounded nerves in animal models [12, 13]. Al-azab et al in their research on the sample of 120 patients with TN have found that LLLT is effective in reducing pain and improving amplitude of compound muscle action potential of temporalis and masseter muscles [14]. Antonić et al. found that LLLT significantly reduces pain in patients with TN, while 810 nm laser compared to 660 nm laser demonstrates higher pain relief [15].

Research on animal models has shown that therapeutic ultrasound (TU) produces a long-lasting antinociceptive, hypoalgesic effect on trigeminal neuralgia. The two theories explaining the biophysiological effect of ultrasound include thermal and non-thermal effect theory. Thermal effects are generally used to manage pain, muscle spasm, and improve connective tissue disorders in sub-acute and chronic conditions [6]. Hsieh YL in his study reported that TU modulates pain by reducing the nitric oxide synthase expression in the spinal cord [16]. Savernini et al in their research have concluded that antinociceptive effect of TU occurs by an opioidergic mechanism [17]. Therapeutic

ultrasound used in rehabilitation treatments utilises frequencies within 1–3 MHz with intensities of 0.1–2.0 W/cm2. TU is most commonly applied on the anterior to the opening of ear canal where the nerve is superficial and devided into three branches [6]. Low-intensity therapeutic ultrasound device that patients could self-apply and operate during daily activity for up to 6 h has been developed and applied in clinical settings with positive effect on pain reduction [7]. Phonophoresis is a process of local administration of topical medicines under the influence of ultrasound. Thermal, non-thermal, and chemical effects generated by the ultrasound, drive the drug molecules into the tissues causing an enhanced penetration [10,13] The use of ultrasonic waves to induce topical medicine is considered painless, noninvasive and has fewer side effects as it administered locally at the site of pain [14]. Commonly used drugs in phonophoresis for rehabilitation purpose are usually anaesthetic or anti-inflammatory agents such as lidocaine, salicylates, hydrocortisone, and cortisone which are targeted locally at the pain site [4, 6]. The effectiveness of TU in the treatment of TN has been confirmed in various case studies, but further research is needed to determine TU place in everyday practice.

Transcranial magnetic stimulation (TMS) is one of the most frequently studied techniques in chronic pain research. This technique uses a magnetic field, inducing an electrical current in the underlying brain tissue. Repetitive Transcranial Magnetic Stimulation (rTMS) modulates neural activities not only in the stimulated area, but also in remote regions that are interconnected to the site of stimulation. The effects of rTMS have been examined in the treatment of various conditions such as major depression, chronic pain, tinnitus, obsessive compulsive disorder, and movement disorders. Studies have shown that prolonged pain relief can be obtained by repeating rTMS sessions every day for several weeks at 10 Hz frequency [7, 19]. Seada et al. in their research concluded that repetitive transcranial electromagnetic stimulation at 10 Hz and 50 mA, for 20 min is considered more effective than low level laser therapy at reducing trigeminal pain, and improving the maximum mouth opening, and masseter and temporalis muscle tensions [19]. A different investigation included 12 patients who failed surgery with intractable TN, out of which 58% reported more than 30% reduction of pain intensity following rTMS [20]. Khedr et al. designed a study in which TN was treated with daily 20-Hz motor cortex stimulation over the course of five days. Ratings of pain decreased by 45% for at least 2 weeks [21]. rTMS is considered as useful and safe modality in the treatment of TN.

#### Conclusion

Treatment of TN is very challenging, because individual responses to different therapeutic options may vary considerably among patients. Only a few available therapeutic options have shown enough efficacy to fulfill current standards for evidence-based medicine. However, there are many novel therapeutic options; physical therapy modalities are included in clinical trials on larger patient populations specifically for TN. Outcome predictors and risk factors for treatment failure are being systematically assessed so that an individual patient-guided treatment decision can be made. The continual effort by clinicians, researchers, and the pharmaceutical industry may soon provide therapeutic options that are more tolerable, more specific, and more efficient for patients with TN.

Innovative multimodal approaches should be considered for those patients presenting with intractable TN. Multimodal approach includes physical therapy procedures as well as the neuropsychological aspects of pain. This new holistic approach places the patient in the center of a multidisciplinary curing team. To this aim, future studies are needed to better understand the mechanisms related to this disease and the impact of multimodal therapies in pain control and life quality of patients suffering from TN. Physical therapy provides non-invasive and cost-effective techniques that can harmlessly provide analgesia in a difficult to treat TN. Promoting such interventions may prevent unnecessary surgery and excessive use of drugs in patients suffering from TN.

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# SINGLE POSTERIOR IMPLANTS IMMEDIATE LOADING BY MEANS OF ONE ABUTMENT ONE TIME

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#### Abstract:

Immediate loading of posterior mandibular implants is well documented procedure when proper case selection criteria are applied. Immediate loading of single implants can be performed either by means of temporary or definitive abutments. There is a hypothesis that repeated abutment disconnections/reconnections have potential negative influence on peri-implant marginal bone loss changes in partially edentulous patients. This paper will cover scientific and clinical principles of immediate loading of single posterior mandibular implants by means of one abutment at one time protocol, as well as by means of conventional temporary abutment.

Key words: single implant, immediate loading, one abutment one time, temporary abutment, zirconia

In modern implant dentistry, osseointegration and bone regeneration are no longer the main focus. Patients are not only asking for functional restorations but also for natural-looking and aesthetically pleasing implant rehabilitations.

Recent modifications in implants and implant-borne prosthesis designs were associated with the introduction of tapered implants, internal hex connections and platform switching [1]. New restorative options included screw-retained prostheses, thus facilitating retrievability and eliminating cement, but generally required precise implant placement for optimal restoration [2]. Implant loading timing has been tremendously changed too, ranging from conventional to immediate loading [3, 4].

Various surgical and prosthetic protocols used in oral implantology are directly associated with the long-term outcome of implant prosthesis. Different implant placement options have been clinically applied., as well as various loading protocols. Evaluating outcomes in oral implantology by combining the placement and loading protocols are paramount [4].

The moment when implants are put into function is marked by the connection of the implant prosthesis. The choice of the most suitable loading protocol is an important step in the process of treatment planning.

The definition of loading protocols has been slightly modified over the years and is currently accepted as follows [4]:

- a) Immediate loading is defined as being earlier than 1 week after implant placement
- b) Early loading of dental implants between 1 week and 2 months after implantation
- c) Conventional loading of dental implants >2 months after implant placement

A single implant can be used to replace a single missing tooth in in the posterior mandible, providing a secure and stable replacement option. The indications for single implant placement in the posterior mandible assume the presence of adequate bone quality and quantity, properly positioned adjacent dentition, sufficient amount keratinized tissue width, as well as an appropriate restorative space.

If the treatment plan includes single posterior mandibular implant placement and immediate loading of it, there are several factors that should be taken into consideration:

Dental anatomy: The anatomy of the mandible, including the presence of critical structures such as the inferior alveolar nerve, should be evaluated to ensure the safe placement of the implant of adequate implant dimensions.

Quality and quantity of jawbone: Adequate quality and quantity of jawbone is necessary for successful immediate loading. A bone augmentation procedure may be necessary if the jawbone is not suitable for immediate loading. Excessive bone augmentation procedures exclude the possibility and indication for immediate loading

Implant design: The design of the implant, including its length, width, and thread design, should be selected to ensure proper stability and support for the immediate loading.

Loading protocol: The loading protocol, including the type of dental prosthesis used, the number and distribution of abutments, and the type of attachment system, should be carefully considered to ensure optimal stability and longevity of the implant.

Occlusal factors: The occlusal relationship between the implant and adjacent teeth should be evaluated to ensure proper stability and to avoid any harmful forces on the implant.

Patient factors: The patient's overall health, oral hygiene habits, and commitment to regular dental visits should be considered to ensure the success of the immediate loading procedure.

Implant primary stability and insertion torque are treatment regulators related to the surgical technique. These two modifiers are determined at the time of implant placement. In optimal conditions, an implant should achieve its primary stability at insertion. If an immediate loading protocol is selected, the initial stability should range from 30 to 50 Ncm depending upon the implant system. Resonance frequency analysis or RFA is also a method of measuring these modifiers. RFA with values greater than 55 ISQ would be required before considering immediate loading of an implant.

It's important to work closely with a qualified dental professional, who can help you make informed decisions about the best course of treatment for your individual needs. According to the ITI SAC Classification, immediate loading is considered an advanced to complex treatment. For the mandibular molar region, immediate loading is a predictable procedure and can be generally recommended in cases where clinical benefits have been identified.

Immediate loading of single implant crowns can be performed either by means of provisional abutment and provisional crown, or by means of definitive abutment and provisional crown. The latter concept, using definitive abutment at the time of implant surgery and implant loading is known as one abutment one time concept. One of the limitations of the standard prosthetic protocols for implant treatment is the need to disconnect and reconnect the prosthetic components. Such frequent exchange of abutments may disturb the surrounding peri-implant mucosal barrier and subsequently cause marginal bone loss. In the era of increased emphasis on minimizing soft and hard tissue trauma, the "one abutment - one time" protocol was introduced as minimally invasive prosthetic protocol. Several publications have documented its positive influence on marginal bone stability over time [5,6].

In implant dentistry, proper distribution of occlusal forces is very important, especially during immediate loading. The main causes of loss of immediately loaded implants are insufficient primary stability and traumatic occlusion. Therefore, it is necessary to establish stable occlusal contacts, as well as harmonious mandibular movements without functional disturbances and occlusal interferences, with the application of the concept of mutually protected occlusion.

It is described in the literature that the occlusion model on implant-supported dental restorations should be designed to reduce occlusal interference, direct occlusal forces centrally along the longitudinal axis of the implant and reduce lateral forces, i.e.to be similar to natural teeth [7,8]. However, it is not known how much we can deviate from these recommendations when designing occlusion models in implant prosthetics. A study examining the influence of occlusal factors on the outcome of implant therapy showed a significant deviation from the "optimal occlusion" that is most often recommended for dental restorations on implants, but without any negative impact on clinically and radiologically monitored variables, as well as patient satisfaction [9].

When planning occlusal restorations on implants, the same occlusal concepts as in natural dentition are applied, even though implants are not, and do not function like natural teeth. Teeth, unlike implants, have a periodontal ligament and their mobility in the vertical direction is 25-100 mm and 56-108 mm laterally, while the movement of osseointegrated implants is about 3-5 mm vertically and 10-50 mm laterally [10–12]. Also, the absence of proprioceptive feedback and the absence of mechanoreceptors means that implants have low tactile sensitivity and may be more prone to occlusal overload [13,14] Implant-protected occlusion aims to create an effective masticatory scheme to reduce undesirable occlusal overload. Clinical functional analysis of occlusion involves analysis of the distribution of occlusal contacts by conventional method, using occlusal markers (articulation paper, foils and special waxes and varnishes). Nonetheless, the application of occlusal markers proved to be an unreliable indicator of occlusal forces because it is based on the dentist's subjective interpretation about the size of the occlusal marker mark on the teeth and restorations (as an indicator of the strength

of occlusal forces)[15,16,17]. Also, conventional occlusion analysis does not have the ability to quantify occlusal contacts. Computer analysis of occlusion, using devices such as T-scan, shows the distribution and intensity of occlusal contacts in real time, both for individual teeth and for the whole dental arches as well [18,19]. Furthermore, this method enables precise locating of the center of occlusal forces (COF).

Implants react differently than natural teeth under occlusal load [11,13, 20]. The periodontal ligament of the natural tooth absorbs the occlusal forces, and then the occlusal load is directed to the crestal bone. Teeth have a similar modulus of elasticity to bone and under the occlusal forces they move in two phases. The first phase occurs within the alveolus of the tooth by stretching the periodontal fibers, and the second phase represents the elastic deformation of the alveolar bone. [20]. Stresses that occur at the junction of implant and bone often exceed physiological ones and can be the cause of peri-implant bone loss [11, 17].

Failure of implant-supported dental restorations has always been associated with biomechanical complications. The factor of occlusal overload can have [21] negative impact on the longevity of the implant. According to Isidor (2006), although the association of excessive occlusal forces with the loss of oral implants is emphasized, the connection has never been convincingly demonstrated [22]. On the other hand, Carlsson (2009) highlights implant loss caused solely by occlusal overload as a dogma that should be abandoned as such[23].Studies that examined the loss of peri-implant bone tissue in conditions of occlusal overload are contradictory. They emphasize that the loss of bone tissue around implants occurs under the influence of large occlusal interferences or in the presence of small disturbances associated with inflammation [24]. Loss of peri-implant bone tissue and/or loss of osseointegration, Lang et al. (2000) associate with biological complications. such as peri-implantitis [25].

Prevention of failure of implant therapy requires checking and analyzing the functional occlusal load of the implant and the surrounding tissue, both in vitro and in vivo. During function, the peri-implant bone tissue is exposed to a constant load. Like all rigid materials, bone is subjected to wear and tear, so repeated pressure can lead to micro fractures in it.

Today, it is believed that changes in bone structure occur as a result of a system in which local mechanical signals cause the activity of bone cells. Frost (1994) proposed a theory according to which there is a minimum level of tension in the bone ("minimum effective strain" - MES), above which adaptive processes take place, while below it the bone remains stable [26]. How much load can implant bear without endangering the surrounding bones, is a question to which there is still no concrete answer [27,28,29,30,31].

There is a general consensus that proper planning and optimal design of dental restorations on implants are crucial, in order to avoid the destructive effect of excessive force on bone and implant system [32].

Implantology would certainly progress greatly if there was possibility to predict how bone and implants would behave if individual patient's unique jaw anatomy, bone quality and strength of occlusal forces were taken into account.

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## DENTAL-PROSTHETIC RESTORATION IN SEMI-MOBILE AND **IMMOBILE GERIATRIC PATIENTS IN CONDITIONS OF COVID-19 PANDEMIC**

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#### Introduction

The World Health Organization has not yet declared that the COVID-19 global health emergency is over. In some regions, the number of positive and diseased patients still shows an increasing tendency, whereas decreasing significantly in some countries. In the Republic of Serbia, cyclical fluctuations and sporadic COVID-19 infection outbreaks are recorded. The presence of some new strains has also been confirmed [1].

During COVID-19 pandemic, the Clinic for Dentistry of Vojvodina ensured full compliance with relevant provisions in line with epidemiological guidelines and adequate preventive measures that

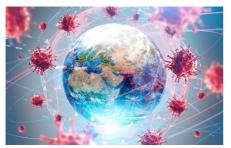


Fig. 1. Earth in Covid-19

were applied to protect patients and professional teams visiting geriatric care centers or performing home visits. Some semimobile and immobile patients received dental-prosthetic treatment at the Clinic of Dentistry of Vojvodina. The rate of human ageing is determined by a wide range of factors. The manifestations of ageing process can be seen in both soft and hard tissues of the oral cavity. Such changes are influenced by numerous factors such as general health condition, dietary habits, maintenance of oral hygiene, as well as the presence and condition of natural teeth [2,3]. Geriatric dentistry strongly requires a teamwork approach. The team includes a specialist

physician, a doctor of dental medicine, medical and dental nurses, and dental technicians. Geriatric patients often manifest with various comorbidities [4,5,6,7], i.e., diseases and conditions that must be taken into consideration when establishing the diagnosis and prescribing therapy. One should evaluate the general health status of the patient, potential vision impairments, neurological disease, loss of hand coordination, psychical condition, and decreased salivation associated with age or due to the intake of a particular medication. The latter can lead to consequent poor hygiene of the oral cavity, xerostomia, ulceration, fungal diseases, massive dental plaque accumulation, caries (especially deep caries), remaining teeth abrasion, tooth



Fig. 2. Old women with parodontopathia

substance fractures, and bleeding. All these can result in parodontopathy, spontaneous tooth loss, or extraction.



Fig. 3.



Fig. 4.



Fig. 5.

#### THE OBJECTIVE OF THE STUDY

This work aimed to ensure adequate protection of semi-mobile and immobile patients and subsequent evaluation of the oral cavity status of geriatric patients based upon the anamnestic data obtained from the patient or his/her caretaker and clinical examination. During the pandemic, dental management and prosthetic rehabilitation of semi-mobile and immobile patients were performed in both Covid-19



Fig. 6. PCR test

positive and Covid-19 negative patients, and testing was performed using the PCR technique. Providing first aid and relieving pain, disregarding the pandemics, was our imperative, and patients should not feel neglected and forgotten.

One of our goals was to improve the quality of life of semi-mobile and immobile patients through a teamwork approach and to provide successful dental management leading to the improvement of the overall health status of geriatric patients in spite of the COVID-19 pandemic.

#### **RESEARCH METHODOLOGY**

PRIMUM NON NOCERE! First, do not harm! The dental management protocol was identical for all patients, stratified according to age. The protocol included immediate PCR testing, evaluation of the documents related to testing, evaluation of anamnestic data, clinical examination of the oral cavity, and obtaining written approval of the epidemiologist and other specialist physicians for performing the dental procedure and prosthetic rehabilitation of the patient [8]. The patients were distributed

into the three groups according to age: group I – 65 to 75, group II – 75 to 85, and group III – above 85. The prosthetic restoration was performed using mobile acrylic dentures – total, partial, and fixed dental prostheses – metal ceramic dental crowns (though a very small percentage). The restoration encompassed a total of 79 patients, 49 (62.02%) males and 30 (37.97%) females.

In 80% of patients of both sexes, the following problems were observed:

Adequate treatment was not available in their place of residence.

Distance of the Clinic from patient's place of residence and additional expenses for transportation

Patients were rejected by their dentist, who refused to examine them and provide prosthetic rehabilitation (due to the lack of knowledge and/or experience how to approach the patient in conditions of COVID-19 pandemic).

The patients were referred to larger health centers with an adequate referral letter, which needed a repeated verification in the place of residence, which posed an additional annoyance and pressure for both patient and caretaker.

60% of dental patients have not received even basic help in their place of residence. Such a discriminatory attitude of individual dentists disabled the dental management of patients in or close to their place of residence, thus increasing the fear and anxiety of pandemics and the dental procedure itself. This could be explained by either poor motivation of the dentists or (more likely) inadequate knowledge and skills in working with geriatric population and semi-mobile and immobile patients during COVID-19 pandemic.



Fig. 8.



Fig. 7. Dental first aid

#### RESULTS

We presented all results obtained during this research, although reporting only positive and successful results of some research has become a common practice among the professional community. We can rarely read about some "less-winning" results undesirable for publication. In fact, such results are of crucial importance and should not be kept hidden – they should be considered an encouragement to further research and can enable comparison of our results with those of other researchers and authors from our country and abroad. This can improve our knowledge and practical work on the dental protection of patients, with the aim of upgrading the health protection of geriatric patients during the pandemic [9]. As aforementioned, our research included 79 patients aged from 65 to over 85, 30 (37.97 %) females and 49 (62.02 %) males.

Before the dental management / prosthetic rehabilitation, the patients reported the following discomfort:

Fear from the pandemic and dental procedure	79 patients
Pain in remaining teeth	59 patients
Jaw joints pain	23 patients
Teeth shifting – parodontopathy	61 patient
Inadequate old denture	49 patients
Impaired or slurred speech	72 patients
Difficulties with consumption and chewing of food	59 patients
Pain in the stomach (associated with improperly chewed food	59 patients
Psychical distress (mostly fear of not-surviving COVID-19) in both patients who tested	79 natients

Psychical distress (mostly fear of not-surviving COVID-19) in both patients who tested 79 patients positive and those who tested negative for COVID-19

After oral-surgical and periodontal rehabilitation, and healing of soft tissues in the oral cavity, dental-<br/>prosthetic management was performed as following:<br/>complete dentures in both jaws48% of patients of both sexescomplete and partial dentures in both jaws25% of patients of both sexespartial dentures in both jaws23% of patients of both sexesfixed dental prostheses –dental crowns4% of patients of both sexes

Table 1

complete dentures in both jaws	complete and partial dentures in both jaws s	partial dentures in both jaws	fixed dental prostheses – metal ceramic dental crowns
48%	25%	23%	4%



Fig. 9. Total denture 45 years old



Fig. 10. Broken lower acrilic prothesis



Fig. 11. New upper dentures



Fig. 12. New partial lower dentures

#### DISCUSSION

The obtained results can be evaluated from different perspectives, such as the aging process, health status, and sociological, epidemiological, and economic factors; however, the elderly population always depends on the attention and care of others, whether their close or extended family members or caretakers in nursing homes.

Since recently, prosthetic rehabilitation in geriatric patients, as a part of dental management, has been encompassing the increasing number of elderly patients. Apparently, our society experiences a growth in the proportion of older persons in the population [10]. In that respect, appropriate education of doctors of dental medicine, nurses, caretakers, and family members is crucial to providing timely and adequate treatment for semi-mobile and immobile patients.

#### CONCLUSION

When speaking of geriatric patients and taking into consideration all data mentioned above, indications, contraindications, needs and possibilities for prosthetic rehabilitation, we came to a disconcerting fact that almost 65% of patients in question are either partially edentulous with unrestored remaining teeth or completely toothless (without prosthetic restoration). Under conditions such as the COVID-19 pandemic, we can tell that health anxiety and concern for one's own health affected almost everybody. At the beginning of the pandemic, even health professionals were confused and trying to fight the epidemic efficiently. Rapid consolidation and implementation of all available epidemiological measures resulted in a substantial level of control of the COVID-19 pandemic. Geriatric patients, both semi-mobile and immobile, have received proper dental care starting from the first contact. Good communication implicated positive attitude at admission and consideration of their experience of difficulties and distress [11,12,13]. Such patients should never get the impression of being socially marginalized, and they must not be! The health management strategies should include mobile healthcare teams, especially in conditions of the COVID-19 pandemic.

Is the pandemic behind us? No, it is not. We still have to wait; one can never be too careful!!! We must be brave and positive when trying to understand this challenging situation and always put our patients first.



Fig. 13. The return of smiles and faith in the future life

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## IN VITRO ACCURACY EVALUATION OF THREE DIFFERENT SCANNING PROTOCOLS

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#### Abstract:

Accuracy of a digital impression is influenced by several factors. The purpose of this study was to identify which protocol produced the most accurate digital impression regarding one tooth - preparation scenario on a typodont. Data were acquired from a single uninterrupted scan with the use of Medit I700 intraoral scanner, a rescanning of the area of interest, or the deletion of the area of interest followed by a rescan. The measured data (IOS scans) were sorted and further analyzed with the help of Geomagic Control X. The "SINGLESCAN" group displayed the best trueness; the "DELETE&RESCAN" group displayed the best precision. Statistical differences were found in the trueness and precision of the IOS scans captured with the three different scanning protocols, but the differences were so small that they could be considered clinically negligible.

Key words: intraoral scanning; accuracy; scanning protocol.

#### Introduction:

The accuracy of an impression, either conventional or digital, is essential for the longevity of the final restoration. The accuracy of a digital impression is determined by assessing two variables that are independent of each other: trueness and precision. The trueness of an IOS determines the ability of rendering a 3D model as closely as possible regarding surface geometry to the real dimensions of the scanned surface [1,2,3]. The precision of an IOS dictates the reproducibility of a scan and, therefore, the predictability of a scan. Intraoral scanners (IOS) are used to directly record the structural surface shape of teeth and related structures within a patient's mouth to obtain a digital impression. Laboratory scanners are powerful machines capable of digitizing gypsum models or conventional impressions with a very small deviation in trueness and precision. [4,5].

The latest scanners provide fast surface recognition and data acquisition but, in most cases, a single scan of an area is not sufficient, since the light emitted from the IOS cannot reach all the surfaces of a tooth, particularly the interproximal spaces [5]. These mesh holes can also be intentionally created by the operator by cutting off sections of the digital impressions to remove unwanted overlapped oral tissues or after adjustments made on the abutments after the initial scan. Some studies have analyzed the influence of digital cutting, rescanning, and overlapping of scanned surfaces on the accuracy of a digital impression [6,7,8], but the results can be inconsistent.

The purpose of this study was to identify which protocol produced the most accurate digital impression of one single tooth preparation, in which data was acquired from a single uninterrupted scan, a rescanning of the area of interest in order to obtain more data, or the deletion of the area of interest followed by a rescan. The null hypothesis was that there would be no significant difference found in the trueness and precision of the IOS scans captured with the three different scanning protocols.

#### Materials and Methods:

On a typodont (AG-3; Frasaco, Tett-nang, Germany) inserted in the mandibular articulation of a dental mannequin (Phantom head PK-2 TSE; Frasaco) a full crown preparation with a chamfer margin was conducted on the left mandibular first molar. The typodont was digitized with the help of a high-accuracy desktop scanner (Freedom HD; DOF) in order to obtain the reference data consisting of

a standard tessellation language (STL) file.With the use of a Medit I700 intraoral scanner, three different scanning protocols were conducted on the typodont. The scanning protocols consisted of:

(1) A single continuous scan without interruption, capturing as many data as possible without overlapping the already registered areas. The scanning path started distally on the left mandibular third molar and followed the occlusal plane until reaching the left mandibular central incisor at the median line, returning on the lingual side of the prosthetic plane, and finally capturing the buccal part of the prosthetic plane (Figure 1a). This protocol was named the "SINGLESCAN" protocol. All subsequent scans obtained by this protocol were grouped in the "SINGLESCAN" measured data group.

(2) A second scan filling in the missing data and overlapping the already captured areas. The rescanning path started distally on the left mandibular second molar and followed the occlusal plane until reaching the left mandibular second premolar, return-ing on the lingual side of the prosthetic plane, and finally capturing the buccal part of the prosthetic plane (Figure 1b). This protocol was named the "RESCAN" protocol. All subsequent scans obtained by this protocol were grouped in the "RESCAN" measured data group.

(3) A third scan in which the first scan was imported, and the crown preparation interest area was trimmed and rescanned. The trimmed area consisted of the prepared left mandibular first molar, the mesial half of the distal neighboring tooth (left mandibular second molar), and the distal half of the mesial neighboring tooth (left mandibular second premolar). The rescanning path started distally on the left mandibular second molar and followed the occlusal plane until reaching the left mandibular second premolar, returning on the lingual side of the prosthetic plane, and finally capturing the buccal part of the prosthetic plane (Figure 1c). This protocol was named the "DELETE&RESCAN" protocol. All subsequent scans obtained by this protocol were grouped in the "DELETE&RESCAN" measured data group.

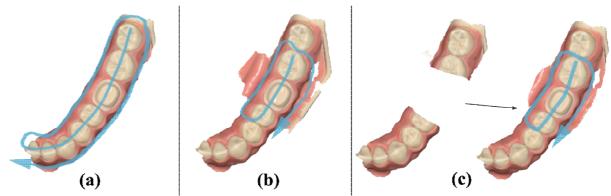


Figure 1. In vitro (a) "SINGLESCAN" scanning protocol path. (b) "RESCAN" scanning protocol path. (c) "DELETE&RESCAN" scanning protocol path.

The scanning scenarios were repeated 30 times following the same protocol, obtaining a total of 90 STL files consisting of three groups of 30 subjects each, representing the measured data for the in vitro part of the study. During the scanning protocol, the typodont-mannequin ensemble was fixed in the same position on the dental chair, the ambient conditions were kept constant, the lighting conditions were kept constant at 1000 lux, and the unit lights were kept off during the scanning procedure. The consecutive scans were conducted by the same experienced prosthodontist with a 10 min break between the different scanning protocols in order to reduce operator fatigue. Any missing data on the scanning mesh was automatically filled by the software by selecting the "Fill Major Holes" feature at the end of each scan. With this feature, based on the "Reliability Map", the reliable areas were extended to cover the areas where data was not acquired.

The measured data (IOS scans) were analyzed with the help of an inspection and metrology software Geomagic Control X (Version:16.0.2.16496, 3D Systems, Wilsonville, OR, USA), in order to obtain the standard deviation for trueness and precision of each scan.

The reference data (laboratory desktop scans) were uploaded into the software and the area of interest was isolated from the rest of the 3D mesh to facilitate the following steps and the comparison protocol. The "3D Compare" function of the metrology software presented the standard deviation results between the measured and reference data via projecting all paired points onto the reference data. The "3D Compare" function also rendered a color-coded map displaying the deviation patterns of the investigated surfaces between  $\pm 0.05$  mm (50 µm). Out-ward displacement is presented on the

color-coded map towards the red spectrum, in-ward displacement is presented towards the blue spectrum, and the green areas where there is less than  $\pm 1 \ \mu m$  difference indicate no deviation (Figure 2).

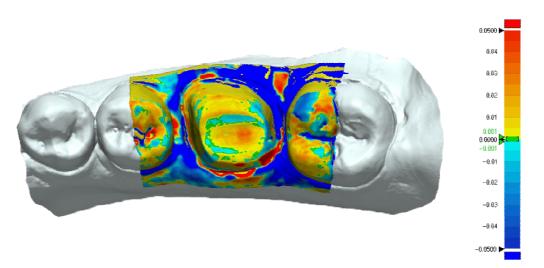


Figure 2. Color coded map of the inspected area of interest, presenting outward displacement (red) and inward displacement (blue) in mm.

To obtain the precision values, the same protocol was conducted by comparing each measured data mesh with all the other meshes within its group.

The organized trueness and precision values were uploaded into the MedCalc statistical software to conduct the statistical analysis. The Kolmogorov–Smirnov test for normality was conducted on the whole set of data and indicated that the trueness values of both in vitro and in vivo cases were parametric while the precision values of both in vitro and in vivo cases were non-parametric. The nonparametric data set underwent Kruskal–Wallis test analysis and post-hoc analysis (Conover test) was also executed. The parametric data set underwent a one-way ANOVA test and the Student–Newman–Keuls test for all pairwise comparisons was also executed. The level of significance was set to  $\alpha = 0.05$ .

**Results:** 

The trueness and precision values of the in vitro case are presented in Table 1.

In Vitno	Trueness		Precision	
In Vitro	Mean SD	Median	IQR	
SINGLESCAN	148.14 µm	3.28 µm	77 µm	15 µm
RESCAN	164.73 μm	9.35 µm	60 µm	8.8 µm
DELETE&RESC	<b>AN</b> 157.66 μm	9.93 µm	56.6 µm	10.6 µm

Table 1. Mean and standard deviation (SD) of the trueness values alongside median and interquartile range (IQR) of the precision values for the in vitro case.

The analysis indicated that there is a statistically significant difference between the trueness groups (p < 0.001). The "SINGLESCAN" group displayed the best trueness, followed by the "DELETE&RESCAN" group with a 9.5  $\mu$ m decrease in trueness, whereas the "RESCAN" group was the least true with a further 7  $\mu$ m decrease (Figure 3a). The overall trueness differences between the analyzed groups were no larger than 17  $\mu$ m.

The statistical analysis of the nonparametric values indicated that there is a statistically significant difference between the precision groups (p < 0.0001). The "DELETE&RESCAN" group displayed the best precision, followed by the "RESCAN" group with a 3.4 µm decrease, and the "SINGLESCAN" group was the least precise (Figure 3b). There was a 20 µm precision difference between the best performing group and the least precise one.

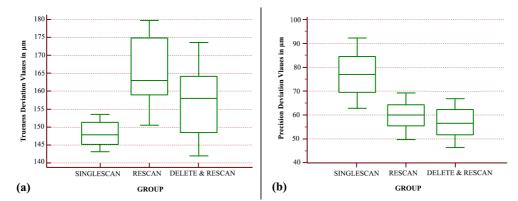


Figure 3. Boxplot displaying the accuracy values. (a) Trueness values. (b) Precision values.

Discussion:

The results of this in vitro study showed that rescanning and overlapping data, as well as trimming and rescanning a surface of a digital model and the different data acquisition protocol, influence the accuracy of the IOS. The results showed that the accuracy values had a similar pattern. The best trueness was displayed by the "SINGLESCAN" group, and the trueness seemed to decrease as more data were overlapped in the other groups. When accounting for precision, the "SINGLESCAN" group was the least precise as there were more surfaces left unrecorded, especially on the proximal areas, during this protocol, in which the holes in the mesh were filled by the software and probably differed between the scans. The "RESCAN" group, having the least unrecorded areas, seemed the most precise.

The overall trueness differences between the analyzed groups were no larger than 17  $\mu$ m, indicating a small difference between the tested scanning protocols. The precision seems to be influenced a greater amount by the scanning protocol as the overall differences reached 35  $\mu$ m.

The location of the evaluated scans was in the posterior arch. This scenario was intentionally selected since lateral proximal areas usually are harder to reach with the IOS's tip and in some cases mesh holes may pass unnoticed.

Miguel Gomez-Polo et al. performed a similar in vitro study using the Trios 3 IOS (3Shape, Copenhagen, Denmark) on full arch scans and with mesh holes of 12 mm placed on the occlusal surfaces of the left second first molar, incisal edges of the central incisors, and the right first molar for one of the test groups, and on the occlusal surface of the left first molar and left second and first premolars for the second test group. They concluded that rescanning mesh holes combined with the stitching software procedures decreased the trueness and precision of the IOS tested [9].

Marta Revilla-Leon et al. investigated the influence of rescanning mesh holes with different diameters (2, 4, and 6 mm) using the Trios 4 IOS (3Shape, Copenhagen, Denmark), showing that the 6 mm group had the lowest trueness and precision. Regarding the influence of rescanning repetitions, they concluded that there were no significant differences between the groups with the same mesh hole diameter. The overall conclusions of the study were that the higher the number and diameter of the rescanned areas, the lower the accuracy [10].

Most of the research states that the clinically acceptable marginal gap falls between 100 and 120  $\mu$ m [11–15]. Based on the approach of the previously mentioned research, we suggest that the resulting trueness and precision differences in the present study, being smaller than 20  $\mu$ m, could be deemed as clinically negligible.

#### Conclusions:

Statistical differences were found in the trueness and precision of the IOS scans captured with the three different scanning protocols, the "SINGLESCAN" group providing the best trueness and the "RESCAN" group providing the best precision. From the three investigated protocols, no specific scanning protocol could be recommended to provide the best overall accuracy.

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# CHAIRSIDE CAD/CAM RESTORATIONS: WHERE IS THE LIMIT?

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# Abstract:

The advancement of computer-aided design and computer-aided manufacturing (CAD/CAM) technologies has a dramatic impact on the fields of prosthodontics and restorative dentistry. Especially with chairside CAD/CAM systems, their popularity has increased dramatically due to the ease of production of indirect restorations. In office CAD/CAM manufactured restorations have many advantages when compared to the conventional method, such as reduced clinical time, good marginal adaptation and color stability, clinically acceptable wear rate and high success rate. The longevity and success rate depends on proper case selection, not every clinical situation being suitable for chairside CAD/CAM dentistry.

In this presentation, a series of clinical cases and treatment planning will be detailed to clarify the limit between chairside and lab side CAD/CAM dentistry. From tooth preparation to intraoral scanning, digital design, milling and cementation, every step should be performed state of the art. What can be expected in the future for chairside CAD/CAM dentistry? Improvement in materials, techniques, and concepts. The evolution of digital technologies will expand the applications, but only a good clinical decision and a patient-centered treatment plan can lead to long-term success.

Key words: intraoral scanning, CAD/CAM materials, properties, indications.

# Introduction

The emergence of CAD / CAM technology in dentistry field has its origins in the end of XXth century, with the idea of using optical instruments to develop a system of layered intraoral mapping of dental surfaces. François Duret, with the opto-electronic impression launched in 1984 the first computerized system for prosthetic restorations [1]. The first genuine CAD/CAM office system belongs to Mormann and Brandestini, who combined the optical impression system with a design software and a milling machine, thus creating CEREC 1 [1,2].

Intraoral scanners (IOS) technology has reached a point where their impact in dental treatments cannot be denied. The advantages that these devices bring to the table when compared to the conventional ways of recording the intraoral structures can be seen in the working speed, the comfort for the patient, intraoperative assessment of the preparations, significant decrease of cross infections and distortion of the impressions and the possibility of indefinite storage of digital models [3-5]. When combined with a CAD/CAM (Computer-Aided Design/Computer-Aided Manufacturing) software, the applications of IOSs range from single tooth prosthetic restorations [6-8], fixed partial dentures [9,10], implant supported restorations and even removable dentures [11,12] (scanning distance influence)

When choosing an office CAD/CAM system there are generally two paths to follow. The first option involves the IOS and the data acquisition software but without any CAD software, therefore the clinician can only take the digital impression that will be sent to a dental laboratory which will complete the remaining procedures. On one hand, this alternative is less time consuming for the clinician but on the other hand, there is significantly less control over the outcome of the restorations. The second option are the IOSs systems that allow full chairside treatments regarding the digital impression procedure, the design of the restorations, milling and depending on the type of material used, sintering and/or glazing of the final restoration.

The greatest benefits of full chairside CAD/CAM systems are the flexibility, speed and treatment predictability. However, the time spent on each case as well as the financial investment can be considerable [13].

# 1. Indications for single visit restorations

The focal point of fixed prosthodontics has changed considerably after the clinical implementation of digital technologies. Minimally invasive treatments supported by the clinical long-term success of bonded CAD/CAM ceramic restorations are becoming the first treatment option for every patient combined with defect-oriented restorations and less conventional preparations [14,15].

Even if nowadays digital dentistry is present in almost all branches of dentistry, still the indications of chairside CAD/CAM systems revolve mostly around cases that can be solved in one appointment. This means that in a matter of hours, the patient can have an indirect prosthetic restoration, therefore removing the conventional waiting times. The most suitable single unit restorations are represented by crowns, but also partial coverage restorations like inlays, onlays and veneers. However, the involvement of the dental laboratory can greatly expand the field of digital prosthetic treatments including complex full arch rehabilitations, long span restorations, surgical guides as well as a wider variety of materials [16].

Minimally invasive treatment options for single unit restorations, like veneers, inlays, onlays, endocrowns (Fig. 1, a,b) and table tops (Fig.2 a,b) are the indications of choice for chairside CAD/CAM systems.

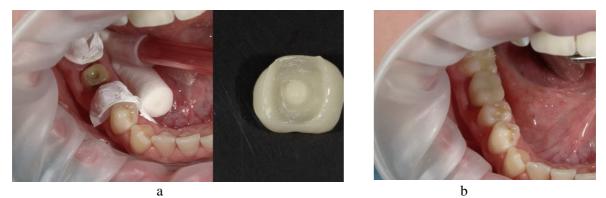


Fig.1a. Preparations for endocrown on tooth 46, endocrown milled from Vita Enamic block.b. Endocrown adhesively cemented in place.



Fig.2a,b. Table top from Cerasmart GC block, before and after adhesive cementation.

# 2. Material selection

Apart from an accurate digital impression which allows an adequate design of the final restoration with optimal marginal and internal fit, an equally important step is material selection. Depending on the type of prosthetic restoration, the materials used can dictate the long-term success of the treatment. Nowadays, the range of materials that can be milled has greatly increased, ranging from nanoceramics, to glass ceramics, lithium disilicate and zirconia. Posterior restorations imply greater mechanical properties to withstand the occlusal loads, while the anterior arch is more esthetically focused. These general rules need to be corroborated with the particularities of each case to achieve the best possible results [17-19].

### 2.1. Characteristics of materials for chairside restorations

Knowing the mechanical properties of each dental restorative material is essential for case selection and treatment planning and with the main focus on minimally invasive restorations, ensuring an optimal thickness for the milled restoration is mandatory. Intraoral factors such as humidity, pH or temperature from oral cavity can alter dental material characteristics. This can lead to mechanical damage, appearance and propagation of cracks, and resistance reduction over time [20].

The two most important characteristics of CAD/CAM materials are resistance and translucency. As much more resistance is a material as less esthetic is. From feldspathic ceramic to zirconia it is an ascendent curve regarding the resistance and a decreasing in translucency.

# 2.1.1. Strength

One of the studies conducted in the Department of Prosthodontics, Faculty of Dentistry Timisoara, evaluated the resistance of thin occlusal veneers milled from three different chairside CAD/CAM materials: a nanoceramic resin (Cerasmart, GC Europe Dental Products, Tokyo, Japan), a lithiumdisilicate-strengthened lithium aluminosilicate glass ceramic (Straumann Nice, Freiburg, Germany) and a composite resin (Tetric CAD, Ivoclar Vivadent, Schaan, Liechtenstein). Ninety intact maxillary human molars with average dimensions of  $11.5 \pm 2$  mm bucco-lingually and  $10.0 \pm 2$  mm mesiodistal crown width, were selected. The occlusal enamel of each tooth crown was removed, the fissures were enlarged, and the sharp margins were rounded off to simulate tooth wear. Part of the specimens that were not selected to be exposed to acidic artificial saliva (n = 30) were stored in physiological serum to avoid desiccation and the saline solution was changed once a week throughout the study. The other remaining part of specimens (n = 60) were immersed in acidic artificial saliva, for 1 month, at 37 °C±1 °C. Using the intraoral scanner (PlanScan, Planmeca, Helsinki, Finland), a digital impression of each prepared tooth was taken. Using the PlanCAD® Easy (Planmeca-www.planmeca.com), the restoration design was made. Occlusal veneers were fabricated with a milling machine (PlanMill 40, Planmeca, Helsinki, Finland). The restorations were milled with a thickness of 0.5 mm and cemented with an adhesive dual-cure resin cement (Variolink Esthetic DC-Ivoclar Vivadent, Schaan, Liechtenstein).

To determine the static fracture load, on both groups of specimens (exposed and non-exposed to acidic artificial saliva), a vertical compression test was performed, using a universal testing machine (Zwick Proline Z005, Ulm, Germany).

From the non-exposed specimens to acidic artificial saliva, the most resistant out of the three tested CAD/CAM restorative materials was Cerasmart with a mean value of 2131 N followed by Straumann Nice (mean value of 1919 N) and the least resistant, Tetric CAD, with a mean value of 1413 N. In the exposed specimens to acidic artificial saliva group, the most resistant CAD/CAM restorative material was Cerasmart (mean value—1333 N), followed by Straumann Nice (mean value—1313 N) and Tetric CAD, with a mean value of 1135 N.

# 2.2.2. Translucency

Another study conducted in our clinic investigated the effects of surface topography and the mechanical features of different restorative computer-aided designs and computer-aided manufacturing (CAD-CAM) dental materials after exposure to simulated gastric juices. Forty disks (n = 10/subgroup) of feldspathic ceramic (Triluxe Forte, VITA, Zahnfabrik), nanoceramic resin (Cerasmart, GC Europe), hybrid ceramic (Enamic, VITA, Zahnfabrik), and leucite-reinforced glass ceramic (Empress CAD, Ivoclar, Vivadent) were used in this study. CAD-CAM blocks were milled into disks (1 mm thick, 14 mm long, and 12 mm wide), with a precision saw and finished with abrasive paper (using different granulations (P240, P400, P800, P1000, and P1200). All analyses were carried out before and after gastric acid exposure. Specimens were individually immersed for 18 h in 5 mL of simulated gastric acid solution and stored in an incubator (Cultura, Ivoclar, Zurich, Schwitzerland) at 37 °C.

Color parameters were analyzed using a spectrophotometer (VITA Easyshade V, Bad Säckingen, Germany). The total color change ( $\Delta E$ ) was calculated for each monolithic material using the following equation:

$$\Delta E_{ab} = \sqrt{\left[ \left( \Delta L^{*} 
ight)^{2} + \left( \Delta a^{*} 
ight)^{2} + \left( \Delta b^{*} 
ight)^{2} 
ight]}$$

Translucency is the ability of a material to transmit light and it can be calculated by the translucency parameter (TP), applying the following equation:

$$TP = \sqrt{\left(L_B^* - L_W^*\right)^2 + \left(a_B^* - a_W^*\right)^2 + \left(b_B^* - b_W^*\right)^2}$$

The results of the study showed that the translucency parameters of the disks did not suffer any drastic changes after 18 h of immersion, for any of the tested materials, as can be seen in Figure 3.

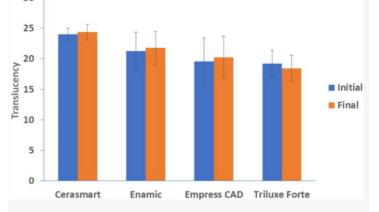


Fig.3. Translucency values before and after acidic exposure of the investigated CAD/CAM materials.

### 3. Conclusion

Strength evaluation of thin occlusal veneers from different CAD/CAM materials, before and after acidic saliva exposure showed that the investigated 0.5 mm thickness occlusal veneers from CAD/CAM restorative materials showed a higher compressive load, compared to those immersed in acidic artificial saliva and/or submitted to thermal cycling process, values which exceeded both normal and parafunctional bite forces, including the specimens immersed in acidic artificial saliva. However, the SEM images showed that the acidic artificial saliva and thermal cycling process influence the restorative materials resistance. It was obtained a failure degree of I and II in the case of the specimens which have been only thermo-cycled, meaning only extensive cracks and fracture of the restorations, while the samples which were immersed in acidic artificial saliva and submitted to the thermal cycled study, present longitudinal and profound fractures of both restorations and tooth. Anyway, in case of any CAD/CAM restorative materials presented in this study, no fracture involved pulpal tissue

From the non-exposed specimens to acidic artificial saliva, the most resistant out of the three tested CAD/CAM restorative materials was Cerasmart with a mean value of 2131 N followed by Straumann Nice (mean value of 1919 N) and the least resistant, Tetric CAD, with a mean value of 1413 N. In the exposed specimens to acidic artificial saliva group, the most resistant CAD/CAM restorative material was Cerasmart (mean value—1333 N), followed by Straumann Nice (mean value—1313 N) and Tetric CAD, with a mean value of 1135 N.

Regarding the effects of surface topography and the mechanical features of different restorative computer-aided designs and computer-aided manufacturing (CAD-CAM) dental materials after exposure to simulated gastric juices, Triluxe Forte was the CAD-CAM monolithic restorative material that suffered the most important changes after exposure to simulated gastric acid solution (i.e., decrease of hardness, increasing roughness, color change, appearance of pores and irregularities and visualization of crater-like grooves, which means degradation of ceramic part or other components embedded into the material). On the other hand, the Cerasmart monolithic restorative material was proven to be the least affected after simulated gastric acid exposure.

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# DENTAL PLAQUE AND SALIVA MICROBIOME. DO WE KNOW ENOUGH ABOUT THEM?

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### Abstract:

Recent studies have revealed the presence of a complex community in the oral cavity. However, the community of microbes in dental caries and periodontal diseases and its interaction with associated bacteria is not yet clear. Fungi and bacteria often engage in complex interactions, such as the formation of multicellular biofilms within the human body. Knowledge about how interkingdom biofilms and microbiome in saliva initiate and coalesce into higher-level communities and which functions the different species carry out remain limited. Scientific research suggest that further work is needed to ascertain the significance of oral mycobiome members.

Key words: dental plaque, microbiome, saliva, inerkingdom interaction

### Introduction

The primary role of bacteria within dental plaque and saliva in the etiology of dental caries and periodontal diseases is well established. However, the specifics of this role have been constantly changing and evolving. The microbial life on Earth often residers on surfaces, where cells form multicellular structure known as biofilms (1). Extensive efforts have been devoted to understanding the biofilm formation process and the mechanisms underlying the biofilm lifestyle (1-3). Most studies have focused on bacteria but there is recent emergence of studies on oral fungal communities-the oral microbiome. Furthermore, these studies have relevated that biofilms composed of bacteria and fungi are highly abundanced in nature, establishing complex interkingdom interactions (4-7). Such bacterial-fungal biofilms can display enhanced virulence and survival, which is achieved throught tigh cell-cell cohesion, metabolite exchange, and extracellular polymetric matrices within established communities (4-6). In the human oral cavity, biofilms formed by bacteria and fungi have the major impact on health (7, 8). How interkindgom biofilms initiate and develop on the surface, and which functions the different spacies carry out during this process, remains still unclear.

# Oral cavity

The oral cavity is home to one of the most complex, and dynamic and diverse microbial collection in the human body. This collection, termed the "oral microbiome", comprises mainly bacteria, viruses, fungi, protozoa and archaebacteria. It is also the oldest recognized microbial ecosystem. Current estimates suggest that up to 1000 bacterial species are present in the mouth, inhabiting several distinct microbial niches. These inscudes saliva, the teeth, the gingival sulcus, the attached gingiva, the tongue, the cheek, the lip and the hard and soft palate (9). Within each oral habitat, the microbes can be found growing in a distinct community, or biofilm, a functionally and structurally organized, matrix-enclosed aggregate of microorganism's which adheres to surfaces such as tooth enamel. That oral microbiome is readily accessible and easily sampled has resulted in it being the most-studied human microbiome and it serves as a model for biofilm biology in general (10-14). One of the major challenges facing oral health researchers today is distinguishing which of the potential host-microbial interactions are critical for the maintenance of dental health. Defining the composition of the oral microbiome is the first logical step in achieving this goal by providing essential information for future metagenomic and metabolomics studies correlating microbial community composition and metabolism with health and caries and periodontal status (15).

### NGS (new generation sequencing)

Advanced in DNA sequencing and bioinformatics have facilitated the disclosure of associations of numerous oral bacterial taxa with dental health or with caries and periodontal activity (16). The use of next-generation sequencing (NGS) technology has revealed the complexity of microbiomes at unprecedented levels and is providing a foundation to understand how hundreds of bacterial species coinhabit and functionally interact to maintain homeostasis, to discourage the establishment of pathogens, and, when conditions are favorable, to cause disease (17). Typically, studies using NGS to characterize microbial communities target one or more regions of the 16s rRNA gene, which due to their hypervariability serve as good markers or bacterial taxa in samples (18). This approach has recently been used in a series of studies to explore microbiomes of dental caries (19), providing better insight into the diversity of the microbial community associated with dental caries. Most research investigating the oral microbiome focuses on bacteria only (20), with over 700 bacterial taxa known to be present in oral cavity of humans (10). Currently, we little known about the contribution of fungi to the oral microbiome and their role in oral health as well as dental caries. In the past decade, more than 20 original research articles have explored the composition of oral microbiome in oral samples. All these studies relied on amplification and sequencing of the ITS region, which is located between the 18S and 28S ribosomal RNA (rRNA) fungi genes and includes ITS-1 and ITS-2 region.

### Microbiome. Do we know enough?

In the human oral cavity, biofilms formed by bacteria and fungi have the major impact on health (7, 8). For example, patients affected by severe childhood caries (tooth decay), a widespread and costly infectious disease affecting toddlers worldwide (21) display high carriage of the bacterium Streptoccocus mutans and the fungus Candida albicans, both in saliva and in biofilms formed on teeth (dental plaque) (22). Previous studies have shown that these distinct microbes from interkingdom biofilms with enhaced virulence under sugar-rich conditions. However, ineractions of these two species in saliva have not been characterized, and the extent to which the interaction between S. mutans ans C. albicans influence the dynamics of biofilm formation and its functional properties is still unknown (23). Microbial surface colonization is a critical step for the biofilm formation, which requires microorganisms to tackle a range of environmental stresses to ensure surface binding, survival, and growth for successful community establishment. Scientific research reveal an underkingdom bacterial-fungal assemblage in human saliva that behaves as a single organismal entity with emergent functions and new spatial growth mechanisms (23). This dynamic interactions C.albicans and S. mutans lead to biofilm superstructures that cause extensive and more severe damage of the tooth-enamel surface and a unique mechanism of microbial migration and biofilm spatial expansion with disease implications (23). These findings suggest that taxonomically distinct microorganisms can organize into biostructures that display supraorganism-like properties, wich refers to interacting individuals that behave in concert as a single unit with enhanced functions in analogy to a comlex higher organism (23). An interkingdom symbiotic assemblage is found in human saliva that behaves like a supraorganism with emergent functionalities to enhance surface colonization, survival, and microbial growth dynamics (23). These observations could be of clinical importance to provide insights into the onset to severe childhood caries characterized by rapid and aggressive decay of the tooth enamel. But most studies involving early childhood caries (EEC) were largely focused on oral bacteria or pathogenic C.albicans. But the newest articles noticed the novel correlation between integral oral mycrobiome and microbiome, figuring out potential taxonomic biomarkers which may lead to different oral health conditions in children (24). The fungal community was found distinguished into five types on behalf of different oral health status and significantly affected the bacterial profile (24). By analyzing correlation between enriched taxa and carious indexes scientific found that Neocosmospora and Fusobacterium could be considered as potencial biomarkers of good oral health and caries risk, respectively. Numerous studies have investigated the changes of the oral bacterial community in caries children compared with caries free (CF) children, detecting decreased bacterial diversity as well as identifying certain discriminatory taxa including Streptococcus, Prevotella, Veillonella, Neisseria and Rothia that were associated with caries (25). As mentioned earlier in recent years, researchers have gradually attached more attention to the relationship between

oral fungi and caries development. Most ITS-based investigations were focused on the dental plaque, revealning an increased fungal load, decreased community diversity and enrichment of several taxa including C.albicans, Candida dubliniensis, Candida sake, Cryptococcus neoformans and Nigrospora oryzae in samples from subjects with caries while Malassezia globose, Bipolaris sorokiniana, Mycosphaerella and Trichosporon were more relevant to CF status (26). Fungi have been detected in subgingival plaque, but their role in these communites is still unclear (27). The only evaluation of the subgingival mycrobiome using ITS sequencing was performed in HIV-positive subjects on antiretroviral therapy (28). This study found periodontitis was associated with lower mycrobiome diversity ad changes in relative abundance of only 2 taxa – reduced Saccharomyces cerevisiae and increased proportions of a Filobasidiales species- compared to individuals with no/mild disease. A metagenomic evaluation of subgingival plaque has shown C. albicans is enriched in periodontal health (29). The subgingival presence of Candida, however, has been hypothesized to contribute to the process of periodontal dysbiosis by facilitating biofilm formation by bacterial plaque constituents such as Fusobacterium nucleatum and Porhyromonas gingivalis or by allowing bacterial tissue invasion. (28).

# Conclusion

Since van Leeuwenhoek initial discoveries in 1648, the fundamental microbiological question remains "what is there?". Most often, the answer has been simple and rather opportunistic, resulting in as yet to be cultivated bacteria and fungi and those that are present in low numbers being overlooked or ignired. Molecular methods have yielded a more comprehensive picture of composition and diversity of the oral microbiota. However, these methods have also resulted in a deluge of data, leading to a need to develop increasingly sophisticated computational and statistical tools in order to analyse and make sense of it (30).

Further research is needed to reveal why particular bacteria and fungi are present and more importantly, what in fact they are "actually doing there". Understanding how bacteria and fungi interact with each other and their environment in an open system such as the oral cavity is key to ultimate aim of unravelling the complexity of the oral microbiome and to providing a better basis for understanding the important role oral bacteria and fungi play in both oral and general health and disease.

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# USE OF PIDAQ IN A SOCIODENTAL APPROACH FOR ESTIMATING ORTHODONTIC TREATMENT NEED

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### Abstract:

A common way to prioritize orthodontic treatment at government funded health services is by using normative need indicators. Assessment of such expert-based indicators neglects patients perceived need. Consequently, those who experienced high impacts on their oral health related quality of life from their malocclusion were similarly prioritised for treatment as those with lower impacts. This calls a need for a more targeted approach for prioritising treatment to those who have the greatest need and would benefit most from treatment. This paper aims to discuss on the sociodental approach in needs assessment to provide a more comprehensive assessment of needs.

*Key words:* Normative need; impact related need; propensity related need; index of orthodontic treatment need; psychosocial impact of dental aesthetics questionnaire

### Introduction

In Malaysia, orthodontic treatment is provided in government dental clinics under the Ministry of Health Malaysia, in public and private teaching institutions, and in private practices. Treatment at government clinics or public teaching clinics are highly demanded due to their subsidized rates [1]. The demand for orthodontics at government clinics continues to rise as evidenced by the growing number of new patients who request for treatment. On the other hand, the number of orthodontic specialists has not seen a proportional increase with this demand due to the limited slots for orthodontic training and rationed federal scholarships to support the orthodontic trainees [2]. Inevitably, patients would need to wait for long periods of time before they can be treated by the limited number of orthodontic specialists at government facilities [3]. Therefore, the ability to estimate treatment need at the individual and population level can facilitate planning and allocation of resources, as well as assist in efficient expenditure. Hence, careful considerations are required to define need.

"Need" can be broadly expressed into four categories [4]: (1) normative need, in which the need is defined by experts where ideal standards are outlined and need is considered to exist when one falls short of the standards; (2) felt need, or "want" as measured by one's perception of their self-assessment of state; (3) and when action is taken on the "want" by seeking assistance, it becomes expressed need, or "demand"; while (4) comparative need exists for people who are not receiving care compared to others sharing similar characteristics but are receiving care. The definition of need was further extended such that it should also look at the ability of the patient to self-care so that they are able to maintain their state of health after receiving treatment [5]. Therefore, they are the ones who are considered to benefit from treatment.

Assessment of orthodontic treatment need to facilitate treatment priority in Malaysian government healthcare facilities is generally based on the Index of Orthodontic Treatment Need (IOTN) [3, 6]. It is an assessment of normative need, which is defined by experts or professionals where ideal standards are outlined and need is considered to exist when one falls short of the standards [4].

The use of normative need has its short-comings: (1) it assumes the standard of care acknowledged by clinicians should be the norm; (2) it accepts malocclusion as an abnormality when malocclusion is merely a description of features that deviates from normal or ideal occlusion [7, 8]; (3) normality and

abnormality of the occlusion is actually a subjective concept where acceptable and unacceptable occlusion is influenced by psychosocial factors, which are not measured by the instrument [9]. Therefore, depending on normative need assessment alone is not an ideal measure of orthodontic treatment need when the determinants for treatment need and demand not only includes measures of malocclusion but also measures of psychological and social factors due to the impact of malocclusion [10].

Patients have their own reasons for seeking treatment. A local study reported that their main reasons for seeking treatment included to improve their dental appearance (65%) [11]. They also reported that the most important motivating factors for treatment were to have straight teeth (48%), to improve dental health (20%), and to enhance their self-confidence (20%). Improving speech (2%) and chewing (2%) were the least motivating factors. In terms of expectations after orthodontic treatment, 75% believed they will have improved self-confidence, 64% believed they will have an improved social life, and 43% thought they will have better career opportunities.

A broader perspective of 'need' known as the sociodental approach has been advocated for a more comprehensive assessment of orthodontic treatment needs (See review by [12]). This approach involves integrating three levels of need [13]. First, the normative need detects dental disease/abnormality. The second level assesses patients' perceived need. This emphasizes on subjective well-being and recognizes the importance of patients' feelings. It is measured using a patient-centred oral health related quality of life (OHRQoL) instrument that is valid and reliable to record the status of the dental patients' well-being directly from the patient [14]. However, as emphasized by Reissmann (2021), the measures for the patients' perceived need should be based on specific requirements to derive meaningful results. The third level considers need in terms of the ability to benefit from treatment. It is based on the premise that patients' behavioural propensity can affect treatment outcome, thus should be considered in the assessment of need [5].

### Sociodental approach in estimating needs

The concept of sociodental system emphasizes the importance of subjective well-being and quality of life via improved functioning and psychosocial well-being. It also considers patients' behaviours for optimum treatment outcome [13]. According to Gherunpong et al. (2006b), the sociodental model framework is an evidence-based algorithm that distinguishes three levels of needs assessment: First, clinical impairment is detected through normative needs assessment. This is usually done using clinical measures. Since the assessment is professionally judged, the instrument used should be based on current practice. In Malaysia, the IOTN can be applied. Second, impact-related need integrates OHRQoL with normative need. The assessment of OHRQoL identifies patients with normative need who should be prioritized for treatment. Third, propensity-related need is assessed based on the behavioural propensity to treatment of patients with clinical impairment and whose quality of life was affected by the impairment. It also considers evidence-based treatment guidelines for the most effective and appropriate treatment in the decision-making process. Gherunpong et al. (2006b) recommended that the method to assess for propensity-related need should be based on local circumstances.

This sociodental approach has not yet been adapted for the orthodontic service treatment priority system in Malaysia. The first level that assesses normative need would be easy to administer given that a standard tool, i.e., the IOTN has already been used across publicly funded orthodontic services in Malaysia [3, 6]. The primary challenge to introduce the sociodental approach for assessing orthodontic treatment need would be at the second level, i.e., the perceived need due to the lack of a valid and reliable OHRQoL instrument for use by Malaysian adolescents that was specifically developed to measure impacts caused by malocclusion. The Malay version of the Child Oral Impacts on Daily Performances (Child-OIDP) index [15] was a feasible option as previously incorporated by [16]. However, it is a generic instrument that was modified to remove non-useful items and increase sensitivity [14]. Instruments that were developed to address the specific concerns of orthodontic patients such as the psychosocial impact of dental aesthetics questionnaire (PIDAQ) [17], which is also valid for adolescents [18] whom are amongst the most frequent treatment seekers for orthodontic treatment would be the preferred choice.

#### Psychosocial impact of dental aesthetics questionnaire

The PIDAQ measures impact of dental appearance and arrangement on OHRQoL, which is the most common reason for seeking orthodontic treatment. The original PIDAQ comprised of four domains

with 23 items: the Dental Self-Confidence (DSC) domain measures positive dental concepts and is comprised of 6 items that assess dental appearance; the Social Impact (SI) domain assesses interpersonal sensitivity and is comprised of 8 items that measure anxiety levels towards other people's reaction to the appearance of the subject's teeth; the Psychological Impact domain (PI) is comprised of 6 items that assess negative emotions towards one's dental appearance; and the Aesthetic Concern (AC) domain contains 3 items that assess disapproval of the image of one's exposed dentition [18]. Three of the PIDAQ subscales were developed from scales which were able to discriminate subjects with excellent dental aesthetics and those with only minor irregularities as determined by the IOTN-AC [17]: the DSC was adapted from the Self-Confidence Scale [19, 20], SI from the Social Aspect Scale of the OQLQ, and AC from the Aesthetic Scale of the OQLQ [21, 22]. PI items were developed in addition to the rest of the domains [17]. The PIDAQ was specifically developed to assess perceived need for orthodontic treatment, with potential use for assessing changes to the patient's well-being under treatment, distinguishing patients' and providers' perspectives and values, as well as documenting the impact of orthodontic treatment for health policy discussions and setting of clinical guidelines [17].

PIDAQ was cross-culturally adapted for use by Malaysian youths. Two versions were produced, Malay version since Malays are the majority ethnicity [23] and the Malaysian English version [24] since English is a lingua franca used by most Malaysians. Both versions of the Malaysian PIDAQ (PIDAQ[M]) had one item removed from the AC domain because the item "don't like own teeth on video" was not applicable to more than 10% of the respondents. This is probably because not all Malaysian adolescents at the time own their own devices that could record videos regularly. Therefore, PIDAQ[M] comprised 22-items in total. Recent work had shortened the instrument so it would be practical for use in a busy setting. The simplified PIDAQ comprised two items from each domain, giving a total of 8-items. Two shortening methods were applied: (1) item impact method to form the impact simplified PIDAQ (ISP8) and (2) regression method to form the regression simplified PIDAQ (RSP8). Both simplified versions were valid, reliable, and responsive to change [25].

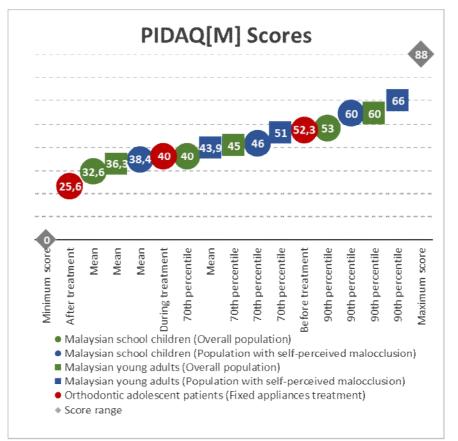


Fig. 1. PIDAQ[M] scores across sub-groups (Reprinted from Wan Hassan 2023)

Application of the PIDAQ across samples of Malaysian secondary school children [26], Malaysians young adults [27] and Malaysian adolescents who sought and had orthodontic treatment [25] showed

that the scores were interpretable with meaningful values (Fig. 1). The minimum and maximum possible scores were 0 and 88, respectively. The mean score of Malaysia school children was 32.6 while the young adults had slightly higher impact at 36.3. The school children and young adults with self-perceived malocclusion had higher impacts with scores of 38.4 and 43.9, respectively. Adolescents who seek orthodontic treatment had a mean score of 52.3, which is between the 70th and 90th percentiles of the populations. These percentiles are considered as values of moderate and severe impacts, respectively [14]. During treatment, the mean PIDAQ scores reduced to a value of 40, which is close to the mean scores of the population. After treatment, the mean scores reduced further at 25.6, which is below the mean score of the population, indicating orthodontic treatment has resulted significant improvement in their psychosocial well-being.

Use of PIDAQ in the sociodental approach to estimate orthodontic treatment need

The sociodental model was tested in a sample of orthodontic seeking patients. The normative need alone found the proportion of patients who needed treatment was estimated as 81.4 to 89.7% [28, 29] depending on the cut-off used for the IOTN.

Initially, a modest cut-off for PIDAQ[M] was applied to assess the impact-related need. This was based on a principle that subjects would be considered to have impact-related need if he/she rated any item as having significant impact i.e., having had the two most severe rating selected. The sociodental estimate that applied PIDAQ[M] as the impact-related need instrument reduced the proportions of those estimated to have treatment need to 65.7% [28].

Later work considered a more stringent cut-off value, in which subjects were considered to impactrelated need if he/she rated any item as having significant impact and had scores that were above the value of moderate impairment [29]. The latter was determined based on the population data [26]. The sociodental estimate of orthodontic treatment need reduced to 35.8% to 39.2%. The use of the ISP8 was not significantly different than when the full-version PIDAQ[M] was used.

# Conclusion

This paper described the work of part of a thesis [30] that was initiated on a hypothesis that the PIDAQ, as a condition specific **Error! Reference source not found.** instrument, would be able to measure from a Malaysian adolescent patients' perspective the impact of malocclusion on their subjective well-being. A series of studies were performed to provide meaningful interpretation to the values of the **Error! Reference source not found.** within the context that it was applied for before the instrument was applied in the sociodental approach for estimating orthodontic treatment need. Finally, the **Error! Reference source not found.** was simplified to promote its potential use in the clinical setting. The outcome established the viability for shifting to a health-oriented model of care that encompasses the expert-defined, subjective well-being, and patients' behavioural aspects of treatment [9]. This will overcome the limitations of a sickness model as used in the current system of orthodontic treatment priority allocation for Malaysian public dental service in facilitating provision of resources.

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# MANAGEMENT OF DEEP CARIES: CHALLENGES AND OPPORTUNITIES

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### Abstract:

Caries prevalence is continuously high all over the world. In contemporary clinical endodontics one of the main goals is preserving the pulp vitality and pulp healthy state and preventing apical periodontitis. Concept of treatment of deep carious lesions has changed considerably over the last decades, and novel methodologies that promote minimally invasive dental procedures have been proposed. Management of deep carious lesions are shifting, with avoidance of pulp exposure. Hereby this paper bring together current evidence regarding the diagnosis and categorization of deep carious lesions.

*Key words: dental caries, minimal invasive dentistry, caries management, selective carious removal, stepwise excavation* 

Introduction

### Etiology of caries

Dental caries is one of the most common chronic infectious oral diseases that results in localized destruction and dissolution of the calcified tooth tissues. Individuals are susceptible to this disease throughout their lifetime, and caries occur at 30-90% of schoolchildren and up to 100% of adults (1,2). Numerous factors influence caries process including diet, oral hygiene habits and fluoride use. Socioeconomic status and genetic factors are also important in caries etiology. Dental caries arises from the complex interaction over time between bacteria from dental biofilm (plaque), carbohydrates and numerous host factors (3). Dental biofilm refers to community of microorganisms adhering to the tooth surface and embedded in an extracellular polymeric matrix. There are several different hypotheses concerning the etiology of caries, which were developed together with research of biofilm pathogenicity. The first hypotheses was the "specific plaque hypothesis", which promote that caries occurs as a result of the action of specific bacteria from dental plaque (4), primarily mutans streptococci and Lactobacillus spp. However, it was observed that the caries developed in the absence of mutans streptococci, and that presence of these bacteria is not always associated with demineralization processes (5). These findings led to the development of the "non-specific plaque hypothesis", which assumes that all plaque bacteria are pathogenic (6). It was observed that certain types of bacteria are more often isolated from carious tissue and that they differ from bacteria found on sound tooth surfaces. This led to the development of the "ecological hypothesis" (7). According to this hypothesis, non-mutant bacteria play a major role in maintaining homeostasis in dental biofilm. Changes in the oral environment, such as frequent intake of carbohydrates, cause ecological disbalance within dental biofilm followed by increase of acidogenic and aciduric species and enhanced production of organic acids. These processes contribute to demineralization finally resulting in a carious lesion (8). Mutans streptococci, lactobacilli and bifidobacteria are recognized as bacteria that initiate demineralization of the tooth surface (9), while in deeper lesion Streptococcus mutans, Lactobacilli spp, Prevotella spp., Selenomonas spp., Dialister spp., Eubacterium spp. and Fusobacterium spp. was found to be prevalent (10). Caries process stimulates pulp defensive reactions, like increased intra-tubular and tertiary dentine formation and initial inflammation.

### Deep caries and pulp diagnosis

Deep caries is defined as "radiographic evidence of caries reaching the inner third or inner quarter of dentine, but with a zone of hard or firm dentine between the caries and the pulp". Therefore, there is a risk of pulp exposure during the treatment (11,12). Deep caries might be further classified into deep and extremely deep caries. Extremely deep carious lesion is defined as radiographic evidence of caries penetrating the entire thickness of the dentine and pulp exposure during the treatment is unavoidable (11,12).

It would be ideal to relate clinical findings of caries with histopathological changes in dental hard tissue, bacterial invasion, demineralization grade, and dentine softness. According to one of adopted clinical classifications, there are soft, firm and hard dentine. Soft dentine is dentine which can be easily excavated with sharp hand excavator or other hand instruments. Firm dentine should be resistant to excavation with hand instruments, while hard dentine is impenetrable by the probe (11,12).

Proper diagnosis is crucial in any treatment. In this case, clinical diagnosis is mandatory to distinguish cases when pulp can be saved with those where pulp cannot be saved. Pulp diagnosis depend on patient's history (chief complain and dental history), clinical examination, radiographic examination and pulp sensibility tests, and is challenging for even skilled clinician (12). It has been considered that there is no significant correlation of signs and symptoms to the histopathological status of the pulp. However, one study found that there is relationship of clinical diagnosis to the histopathological status of the pulp (13). Namely, only 2 of 59 teeth diagnosed as normal or reversible pulpitis had histological signs of irreversible pulpitis, while 5 of 32 teeth clinically diagnosed by irreversible pulpitis had a histological status of reversible inflammation. Patient examination should begin with taking pain history. Pulp sensibility testing, both cold and electrical, in teeth with deep carious lesion reduces risk of incorrect diagnosis. It has been shown that these two tests have good sensitivity and specificity (14), especially when used in combination, correctly identifying up to 92% of vital and up to 96% of necrotic pulps (15). In addition to pulp sensibility testing, allodynia to percussion indicates an irreversibly inflamed state of pulp tissue. Periapical radiographs should be used in order to evaluate precisely the penetration of carious tissue, root formation, etc. Cone beam computed tomography is not recommended for routine assessment of the pulpal state (12).

In order to diagnose the pulpal state, the vital pulp can be diagnosed normal, reversible pulpitis or irreversible pulpitis (symptomatic or asymptomatic), depending on clinical signs and symptoms (12,16). Reversible pulpitis is characterized by lack of symptoms or by the less intensive, short-lasting pain provoked by hot/cold stimuli which resolves after removal of stimuli, and no tenderness to percussion. On the other hand, symptoms in irreversible pulpitis include spontaneous, spreading pain that linger after removal of stimulus and provoke sleep disturbances. It is interesting to note that about 14-60% of cases of irreversible pulpitis are symptomless (17). Reversible pulpitis can be observed as a diagnoses where pulp can be preserved, and irreversible pulpitis where pulp cannot be saved. However, this concept has recently been challenged. Distinct boundary marking possibility of pulpal histological repairment does not exist. Development of vital pulp therapy such as partial and full pulpotomy, aimed to maintaining pulp tissue with irreversible pulpitis in the coronal pulp indicate that there is a need to reconsider classification of pulpitis with inclusion of partial irreversible pulpitis. Partial irreversible pulpitis may correspond better to the histological status of the pulp.

# Treatment to avoid pulp exposure

The strategies of carious lesion management present an important issue in oral health care. In the past, caries was considered a fully infectious disease that can be managed by invasive procedure, entirely removing demineralised and infected tissue. It is an approach where demineralized dentine is completely removed to reach hard tissue, including pulp wall. This treatment approach poses the risk of accidental pulpal exposure. Furthermore, 25-50% of the bacteria remain in the cavity even after complete removal of carious tissue (20). Although minimally invasive biologically based treatments were initiated decades ago, it is still challenged to translate evidence into clinical practice. Questionary based studies highlight evident variation in the treatment of deep carious lesions and absence of standardized decision-making between dentists (18,19). Many dentists worldwide would still choose to carry out complete removal of carious tooth tissue and restore lesions instead of using less invasive management option (18).

One of the main goals of operative dentistry is to reduce intervention and preserve pulp's developmental, defensive, and proprioceptive functions. Research results clearly show that caries can be treated without removing bacteria, but by rebalancing dental biofilm and capturing bacteria in the deep parts of the tissues. Thus, caries process can be regulated throughout alteration of the patient's susceptibility to caries, and inactivation of the active lesion (21). Thus, complete (or nonselective) carious removal treatment approach is considered overtreatment and it is no longer recommended. A biological approach is therefore recommended, including selective carious removal in one or two stages, to avoid pulp exposure often connected with complete carious removal.

One stage selective carious removal or two stage stepwise carious removal are indicated in teeth with deep carious lesions without symptoms or with symptoms indicative to of reversible pulpitis. Also, according to European Society of Endodontology (ESE) Guidelines, it is indicated in cases where deep caries reached "the inner quarter of dentine with a well-defined remaining rim of dentine between caries and pulp" (12). The amount of carious tissue that is removed in less invasive treatment strategies varies between the studies and there is a lack of definition and standardization of the caries removal end point (12). Namely, in some studies the cavity is definitively restored after removing only the undermined enamel without removing the carious dentine (22), while in others after the enamel, the part of the carious dentin is removed, leaving carious mass at the pulpal wall (23). According to recent ESE recommendations, carious tissue should be removed from peripheral enamel and dentine to reach hard dentine (non-selective removal), while leaving soft or firm dentine on the pulpal wall of the cavity only. This approach facilitates adhesive seal of definitive restoration. It is important to note that inadequate restoration and absence of permanent seal during the use of less invasive treatment approaches will result in failure and pulp pathosis. In one stage treatment, after partial carious removal, a definitive restoration is placed immediately (11,12,21). In two stage stepwise carious removal, treatment is performed in two sessions. The first stage includes carious tissue removal to soft dentine and a placement of temporary restoration. After 6-12 months, in the second stage, residual caries is removed completely, as in complete carious removal approach, and restored permanently. It can be viewed as complete carious removal technique in two visits (11,12).

Clinical, microbiological, and radiographic studies have shown that carious dentin that remains in the cavity changes to an inactive form, from wet and soft tissue into a darker, harder and dried appearance with a reduced number of bacteria and increased radiographic density (23-25). It is interesting to note that just sealing of the cavity appropriately even with inert material, might result in inactivation of active carious lesions (26). However, the use of biologically based materials is recommended to be applied before permanent restoration in both one-stage and two-stage procedures. The aim of using biologically based materials is to initiate the formation of tertiary dentine in order to protect pulp tissue from various stimuli and bacterial ingress. Although calcium hydroxide is the most used lining material in deep carious lesion treatments, the recent systematic review and meta-analysis demonstrated that it cannot influence the clinical success of selective removal of carious tissue (27). It is recommended to use glass ionomer or calcium silicate cements (12).

For the appropriate use of selective carious removal techniques, the use of rubber dam and aseptic technique are recommended (12). Carious tissue in selective carious removal is carried out by using round bur or hand excavators. In the ESE position statement, as part of an aseptic protocol tooth disinfection is proposed for deep caries management, but for pulp exposure. Although the use of cavity disinfectants may reduce the bacterial load, there is currently no clinical evidence that it might have a positive impact on treatment outcome. Moreover, they could compromise the bonding strength of adhesive restoration. Therefore, it should be used, if any, after careful clinical consideration (28).

Selective carious removal is superior to complete carious removal, with less pulp exposure and less pain (29,30). When compared two less invasive treatment approach, data showed that the successful outcome of deep caries treatment in two visits was lower compared to one-session therapy after 3 years (69% vs. 91%) (31) and after 5 years (56% vs. 80%) (32). Lower treatment success in two-session therapy might be related to a higher risk of pulp exposure during caries removal in the second visit and bacterial contamination. Also, temporary restoration shows increased microleakage in comparison to a definitive restoration. Some authors point out that the advantages of one-session therapy compared to two-session therapy are the economic side and the less unpleasantness of the procedure itself, because the treatment is completed in the same visit (12). Also, one-surface restorations showed better treatment outcome in comparison to two- or more surface restorations (31).

# Conclusion

Deep caries has traditionally been treated with complete (or nonselective) carious removal. Recent research is supporting the promotion the development of minimally invasive biologically based treatments. A biological approach, such as selective carious removal in one or two stage, is recommended to avoid pulp exposure associated with complete caries removal.

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# DENTURE STOMATITIS AND OTHER CANDIDA–ASSOCIATED INFECTIONS IN THE ORAL CAVITY

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### Abstract:

Despite increasing health literacy and understanding of the need for good oral hygiene worldwide, as well as advances in the development of mechanical cleaning/chemical plaque control devices and improvements in dental materials, opportunistic fungal infections have increased, particularly among wearers of removable dentures. The combination of fungal cell deposition in denture material irregularities, poor oral hygiene, and several systemic conditions are considered to be the main predisposing factors for the development of denture stomatitis and other yeast infections. The most common fungal infection in the oral cavity is caused by Candida species.

Key words: Candida albicans; Denture stomatitis; Oral Cavity Infections

### Introduction

The most common fungal infections in the oral cavity are candidiasis and they are caused by Candida spp., the most common fungi is Candida albicans. It is the most represented yeast on both healthy and infected oral mucosa. Studies show that it is present in the mouth of all humans as part of the normal flora [1, 2].

Conversion to pathogenic flora and the occurrence of opportunistic infection is the result of disturbances in homeostasis caused by various environmental and hereditary factors, which may be local or systemic [2].

Systemic conditions affecting homeostasis include diseases such as cancer and necessary therapy, digestive system diseases, nutritional deficiencies, autoimmune diseases, HIV and primary immunodeficiencies, corticosteroid or antibiotic therapy, pregnancy, and age [3, 4, 5]. Infection of the oral cavity with Canida may serve as an early sign of HIV infection, disease progression, and as an indicator of the immune status of such patients [2, 5, 6].

The described conditions may cause changes in the expression of virulence factors or excessive growth of Candida, as well as disruption of the balance and transition from normal to pathogenic flora [2].

# Candida-associated infections in the oral cavity

Due to the wide spectrum of manifestations of Candida infection, there are several subdivisions. In terms of the color of the change in the mouth, it can be divided into red and white, and according to the origin into primary and secondary. Primary candidiasis refers to infections that affect only the perioral area and oral cavity. However, when the infection occurs in the context of systemic disease and the mucosa is already altered and thus susceptible to infection, it is referred to as secondary candidiasis [2, 7]. Pseudomembranous candidiasis, acute erythematous candidiasis, chronic erythematous candidiasis, and chronic hyperplastic or nodular candidiasis are considered as primary forms of the disease, while angular cheilitis, medial rhomboid glossitis, and chronic mucocutaneous candidiasis are secondary [1, 2, 7].

The primary form, characterized by white plaques on the oral mucosa, is the most common and is called pseudomembranous candidiasis. It is characterized by the fact that it can be scraped off with a light touch, since only the uppermost epithelium layer of the oral cavity is infected, which distinguishes it from other diseases that manifest themselves by white coatings that cannot be removed from the surface. The disease may be localized or generalized and accompanied by poor changes in

taste sensation. It is common in neonates, dry mouth, patients on local steroid therapy, and immunocompromised individuals [1, 2, 7, 8].

The form manifested by red lesions usually on the dorsum of the tongue is called acute erythematous or atrophic candidiasis. The lesions are extremely painful and are accompanied by depapillation of the tongue. It occurs as a result of antibiotics, corticosteroids, and immunosuppressive therapy [1, 2, 7].

Denture stomatitis is the name for chronic erythematous or atrophic candidiasis. The changes are located on the mucosa covered by removable prosthesis, they are red and usually asymptomatic, only sometimes accompanied by a burning sensation [1, 2, 7]

The form in which the white plaques cannot be removed by gentle scratching is called Candida leukoplakia or chronic hyperplastic candidiasis. It may be homogeneous or heterogeneous. Tissue infiltration by yeast cells is deep and considered precancerous, especially in heterogeneous changes. It is most common on the buccal mucosa and lateral parts of the tongue [1, 7].

Cheilitis angularis is a condition with multiple causes, most commonly including anatomic predisposition, xerostomia, immunosuppression, and stomatitis caused by denture replacement, which may or may not be associated with existing oral candidiasis. It is an inflammatory disease of one or, more commonly, both corners of the lips, clinically manifested by redness, erosions, and crusts sometimes covered with white plaques [7, 9].

Medial rhomboid glossitis is an asymptomatic lesion and it is a condition of unknown etiology, often associated with secondary Candida infection [1, 2].

Chronic mucocutaneous candidiasis is a heterogeneous group of syndromes. It most often appears in childhood and is associated with numerous immune disorders [10].

Fungi of the Candida genus are also found on all tooth surfaces - enamel, dentin, and cementum. Candida albicans grows in enamel fissures and grooves and can invade open dentinal tubules [2, 11]. Several species of Candida have been isolated from dentin and root caries in children and adults, with a prevalence of 66-97% in children and 31-56% in adults [2, 11].

### Denture stomatitis

Many people wear certain prosthetic restorations due to partial or complete tooth loss. Denture stomatitis is a very common condition that affects people wearing removable dentures. It can occur in both partial and complete dentures, as well as in the maxilla and mandible. However, studies have shown that the maxilla is most commonly affected in mobile maxillary complete dentures. The etiology of the disease is multifactorial, although it has been demonstrated that Candida albicans is present in over 90% of infection cases. The changes are limited to the area of the mucosa covered by the denture, sometimes they may be accompanied by a sensation of pain, burning, and itching, but they are usually asymptomatic and are detected as incidental findings during a clinical examination [2, 12]. Classification of the disease is based on the appearance of the inflamed palatal mucosa under the prosthesis, and Newton's 1962 classification shown in Table 1 is the most commonly used.

Table	I. Newton's	classification	

0	NO INFLAMMATION
1	POINT HYPEREMIC LOCI
2	DIFFUSE HYPEREMIA ON MUCOSA UNDER THE PROSTHESIS
3	PAPILLARY OR GRANULOMATOUS HYPERPLASIA

Long-term soft tissue trauma caused by unstable and bad-fitting prostheses on the mucosal surface, lesions associated with injuries caused by the roughness and material irregularities of the prosthesis, or patients who have caused injuries due to parafunctional habits belong to Newton's classification type 1. Types 2 and 3 of Newton's classification refer to infections usually associated with Candida, which may occur with or without mechanical trauma [12].

In addition to tissue trauma, hygiene and wearing dentures at night are considered the most important local factors in the development of denture stomatitis. Numerous studies show a clear association between poor denture hygiene and an increased risk of stomatitis. Most patients attempt to maintain denture hygiene only by brushing the denture, just as one would brush the natural dentition, which is insufficient for proper denture hygiene. In addition to mechanical plaque removal by brushing, patients should be strongly advised to use commercially available disinfectant solutions or to soak dentures in diluted sodium hypochlorite as part of routine daily care. In addition to poor hygiene, constant denture wear provides relatively anaerobic conditions and low pH between the denture base and the mucosa, which can promote opportunistic overgrowth of pathogenic fungi such as Candida. The constant wearing of the denture prevents saliva from cleaning the area where the denture is located, which further promotes the proliferation of pathogenic microorganisms. Once microorganisms have settled on the relatively rough surface of the denture, it can serve as a reservoir, and surface irregularities later provide protection for microorganisms and make hygiene more difficult [13].

It is possible to check the degree of hygiene of the prosthesis by staining the base of the prosthesis with eosin and indexing it according to Tarbet (Table 2). The prosthesis is divided into four quadrants and indexed according to the degree of plaque involvement on the surface. In this way, the patient can be shown how well his prosthesis has been cleaned.

Table 2. Tarbet's hygiene index					
0	NO PLAQUE				
1	DISCONTINUED PLAQUE				
2	CONTINUOUS PLAQUE FOR 1/4 QUADRANT INFLUENCE				
3	CONTINUOUS PLAQUE FOR 2/4 QUADRANT INFLUENCE				
4	CONTINUOUS PLAQUE FOR 3/4 QUADRANT INFLUENCE				
5	CONTINUOUS PLAQUE FOR 4/4 QUADRANT INFLUENCE				

In addition to these local risk factors, the occurrence of stomatitis under dentures is influenced by the systemic risk factors already described.

Conclusion

Prevention of Candida infections is of great importance. In the case of denture stomatitis, highquality dentures combined with clear instructions from the dentist for patients about the importance of denture care and daily cleaning are important. Regular examinations at the dentist's office and timely diagnosis are key to reducing infections.

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# EPIDEMIOLOGY OD HEPATIS C IN SPLIT-DALMATIA COUNTY

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### Abstract:

Hepatitis C virus is prevalent worldwide, and the most common cause of high prevalence in intravenous drug use is irregularities in the handling of parenteral interventions in health care facilities. The aim of this study was to determine the prevalence, risk factors, type of infection and distribution of HCV genotypes in different age and sex groups of the population of Split-Dalmatia County in comparison with other counties in Croatia. In Split-Dalmatia County, the predominant cause of hepatitis C infection is genotype 3 (49%), which occurs in intravenous drug users. The association between the risk factors for infection and the distribution of genotypes in a subgroup of patients from Split-Dalmatia County confirms the correlation between injecting drug users and the high prevalence of genotype 3 hepatitis C.

Key words: hepatitis C, epidemiology, Split-Dalmatia County

### Introduction

Hepatitis is an inflammatory disease caused in most cases by the hepatitis virus. In terms of virus type, at least six types of hepatitis viruses are distinguished: hepatitis A, B, C, D, E, and G. All virus types affect the liver, and the basic symptoms are similar, but the virus types differ significantly in their structure, mode of replication, mode of transmission, and duration of onset of the general symptoms of the disease they cause (1).

Viral hepatitis C is an infectious disease caused by the hepatotropic hepatitis C single-stranded RNA virus (HCV) of the flavoviridae family. The virus has been found to be most commonly transmitted parenterally through blood and blood products, and as far as is known, infected humans are the only "reservoir" of the virus and the actual source of infection for other humans. Most people infected with hepatitis C, and it is estimated that there are more than 170 million people worldwide, suffer from a chronic form. Acute infection is usually asymptomatic and often goes unnoticed, whereas in about 80% of people infected with hepatitis C, the disease develops into a chronic form (3).

Today, six major genotypes of hepatitis C viruses are known to physicians and researchers, and within each genotype, a large number of subtypes (more than 90). Genotypes 4 through 6 are restricted to specific geographic areas, while genotypes 1 through 3 are widely distributed throughout the world.

Genotype 2 is found in the Mediterranean region, genotype 3 is common in the population of intravenous drug users, genotype 4 is found mainly in Egypt, while genotypes 5 and 6 are less common. The classification into genotypes and subtypes is clinically important because it can be used to predict the response to therapy and the period of treatment (4).

Hepatitis C has two distinct incubation periods: 2-4 weeks and 8-12 weeks. Research suggests the possibility that there are two different pathogens, one of which is a flavovirus and another of which is a togavirus. Hepatitis C can be distinguished from other types of hepatitis by a high concentration of the liver enzyme alanine transferase in the blood. In all types of hepatitis, various enzymes are released into the blood due to damaged liver cells, but the concentration of this specific enzyme is increased in the presence of hepatitis C virus (2).

Chronic infection leads to serious complications such as liver dysfunction, the development of cirrhosis and hepatocellular carcinoma (HCC) in many patients. Thanks to routine testing of blood from voluntary donors for hepatitis C virus infection, the prevalence of HCV infection has decreased

significantly. Today, the most important risk factor contributing to the spread of hepatitis C virus infection is intravenous drug use (3).

Significantly less is the possibility of HCV transmission in isolated cases of percutaneous exposure during involuntary injections, mucosal contact with infected blood or serum during delivery of an infected mother, or sexual intercourse with a partner (5,6,7).

Hepatitis C virus is prevalent worldwide, and the most common causes of its high prevalence, in addition to intravenous drug use, are irregularities in the treatment of parenteral medications in health care facilities. The incubation period of the virus varies from two weeks to six months.

Chronic infection can last up to twenty years before it leads to the development of cirrhosis or liver cancer. An infected person is contagious one week before the onset of disease symptoms and may remain contagious for life. Susceptibility to infection is universal, and the degree of protection after recovery is unknown. Knowledge of the epidemiology of viral hepatitis is a prerequisite for proposing appropriate disease prevention and control measures. Depending on the most common modes of transmission and the proportion of certain populations among those with the disease, disease control measures can be targeted to newborns, adolescents, and those at increased risk, such as intravenous drug users. When determining measures to prevent and reduce the prevalence of disease, it is important to consider the specificity of one's population.

Croatia is a low prevalence country (less than 2% of the population has anti-HCV antibodies). The epidemiological situation of the selected population determines which part of this population can benefit most from screening and treatment programs (8).

### Research goal

The goal of this study was to determine the prevalence, risk factors, way of the infection and distribution of HCV genotypes within different age and sex groups of the population of Split-Dalmatia County compared to other counties in Croatia.

The aim of the study is to determine the basic epidemiological characteristics of HCV infection among individuals in Split-Dalmatia County. This work will address the following:

- 1. Incidence and prevalence of HCV infection
- 2. Sex and age distribution of HCV infection
- 3. Exposure to risk factors and likely type of infection
- 4. Distribution of HCV genotypes
- 5. Comparison of data on a national basis

Materials and methods

This retrospective study was guided by interviewing of chronic virus hosts about the way of infection and their age when infection was detected. Patients data were obtained from several different sources: the Register of chronic HCV infected of Teaching Institute for Public Health (NZZJZ) of Split-Dalmatia County, register of Infectious diseases department of KBC Split, register of Department of transfusion KBC Split, register of HCV infected patients in the Department of Infectious Diseases from Croatian Institute for Public Health and Croatian statistical year book.

The study includes all registered anti-HCV positive patients from 1993 to 2015. Plasma samples for molecular diagnosis of HCV infection have been collected as a part of routine diagnostic monitoring of the patients with chronic hepatitis C with the aim of determining the effect of therapy. Statistical analysis was performed using the SPSS 17.0.

### Results

In the period from 1993to 2015, a total of 1434 persons with HCV infection were recorded. The range of reported infections is from 23 (in 2014) to 111 (in 2007). Under Diagnosis HCV infections are presented together with anti-HCV positive and HCV antigen carriers (Figure 1).

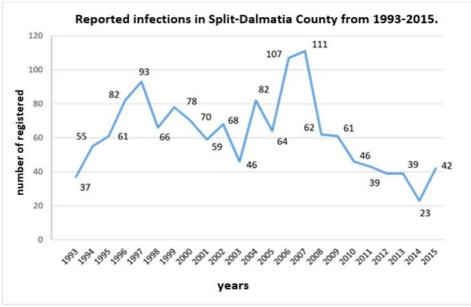


Figure 1. Representation of reported HCV infections in Split-Dalmatia County from 1993. - 2015.

Prevalence of HCV infection in Croatia and Split-Dalmatia County

It is estimated that there are around 40,000 people with chronic HCV infection in Croatia. Considering that Split-Dalmatia County lives slightly less than 11% of the Croatian population, it is estimated that at least 4,000 people have chronic HCV infection (Table 1).

Population category	New voluntary blood donors	Pregnant women	General adult population	Intravenous drug addicts	Children up to18 years of age Total	Total
Population size estimation	9.000	60.000	3.420.000	11.000	800.000	4.300.000
Estimated prevalence of anti-HCV	0,1%	0,5%	0,9%	40%	0,5%	-
Estimation of the number of carriers	9	300	30.780	4.400	4000	39.489

Table 1. Estimate of the number of persons chronically carrying the hepatitis C virus in Croatia

Gender distribution of HCV infection in Split-Dalmatia County

Of the total number of HCV infections, the majority was recorded in men (78%) (Figure 3).

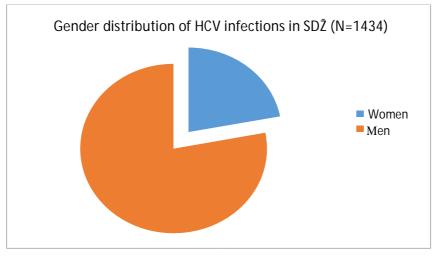
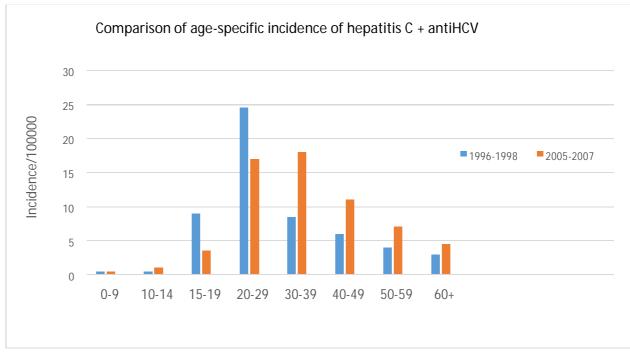
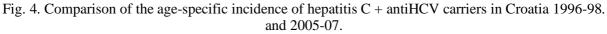


Fig. 3. Gender distribution of HCV infections in Split-Dalmatia County

Age distribution of HCV infection in Croatia

The age distribution of HCV infection in the period 1996-1998 was observed. and 2005-2007. years. In the period 1996-1998, the most reported were in the 20-29 age group, and in the period 2005-2007 the age groups 30-39 and 20-29 are almost equal (Figure 5).





# Conclusions

Based on the research conducted, the following conclusions can be drawn:

1. In Split-Dalmatia County, hepatitis C infection is caused almost equally by genotypes 1 and 3. Men are more often affected, and the most common age at the time of reporting the disease is 20-39 years.

2. The association between the risk factors for infection and the distribution of genotypes in a subgroup of patients from Split-Dalmatia County confirms the correlation between intravenous drug use and the high prevalence of hepatitis C genotypes 1 and 3.

3. The introduction of needle and syringe exchange programs, sex education programs and the possibility of anonymous testing reduces the prevalence of hepatitis C and enables earlier diagnosis of the disease.

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# SOST AND DKK-1 AS BIOMARKERS FOR TREATMENT OUTCOMES OF CHRONIC STATIN THERAPY IN PERIODONTAL DISEASE

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# Abstract:

Periodontitis is an inflammatory process that can lead to loss of alveolar bone strength and loss of teeth. The canonical  $WNT/\beta$ -catenin pathway, along with its inhibitors Dickkopf (DKK-1) and sclerostin (SOST), found in gingival crevicular fluid (GCF), are involved in the processes of bone renewal and healing. Blocking SOST stimulates alveolar bone formation and decreases periodontal ligament space, while DKK-1 has an anti-anabolic effect. The effects of bone morphogenic factor (BMP) on the process of bone growth and development, and the improvement of the clinical outcome of bone mass loss by statin use are important therapeutic targets in periodontitis.

Here, we will discuss their potential as biomarkers for outcomes of chronic statin treatment in periodontal disease.

Key words: periodontitis, DKK-1, SOST, GCF, statins.

### Introduction:

Periodontitis is defined as a microbiological-inflammatory process that leads to clinical loss of periodontal attachment. Formed bacterial biofilm causes inflammation of the gingiva, but the progression of the disease depends on the reaction of the microbiome to the substances from the decomposed and inflamed gingiva. Mediators of the inflammatory process leading to loss of attachment and significant secondary characterization of periodontitis-loss of alveolar bone at the edges. Activation of the host proteinase leads to the loss of the fibers localized in the peripheral part of the periodontal ligament that leads to apical migration of the outer epithelium and thus the apical spread of the bacterial biofilm along the root surface is further enabled. Depending on the number of lesions, oral hygiene, level of oral biofilm contamination, age and whether the patient uses cigarettes or has diabetes mellitus, the severity of the clinical case presentation can be summarized.(1) The new classification of periodontitis distinguishes: necrotizing periodontitis; periodontitis as a manifestation of a systemic disease; and simply periodontitis (this includes forms of the disease previously known as: chronic and aggressive type). In the revised classification, characterization with a multidimensional stage system was agreed upon. The staging system is important for determining the severity of the disease and the complexity of the required treatment, and has four categories numbered from 1 to 4 (determination based on variables: loss of clinical attachment, amount and percentage of bone loss, probing depth, presence and extent of angular bone defects as well as involvement root bifurcations, tooth mobility and tooth loss). The class system distinguishes three levels: class A (low risk); class B (moderate risk); class C (high risk) and other factors, e.g. smoking and diabetes.(2) The established rule for the periodontal patient is oral or buccal clinical attachment loss (CAL) ≥3mm with pockets deeper than 3mm and detected in more than 2 teeth or interdental CAL $\geq$ 2 in non-adjacent teeth.(1) Diagnosing and treating periodontitis is an essential process for the overall health of an individual. Inflammatory processes of periodontia can cause systematic liver diseases, coronary artery disease leading to stroke, or worsening diabetes mellitus type II.(1) The WNT signaling pathway, together with proteins and ligands, plays a role in the processes of renewal and healing, therefore it is important in therapeutic strategies in dentistry. Due to DKK-1 and SOST as inhibitors of this pathway, significant control is made in many diseases such as inflammatory processes of hard tissue and osteoporosis.(3) WNT proteins, cytokines, leukocytes, ions, mediators of inflammatory processes as

well as tissue products were found in GCF. Localized in the sulcus (pocket) between the gingival margin and the tooth, GCF is a serum transudate in healthy gingiva while it is an exudate during inflammatory processes. Considering the treasure of great therapeutic possibilities, the content of GCF can be used for the prognosis and diagnosis of periodontal disease. Complicated sampling is justified by the economic profitability and richness of the sampled content.(4) DKK-1 and SOST are activated inhibitors in periodontitis and proteins found in GCF. DKK-1 has an anti-anabolic effect leading to bone resorption caused by an inflammatory process as well as the healing process of the soft tissue localized in the oral mucosa. Increased SOST blocking narrows the space of the periodontal ligament and stimulates the formation of alveolar bone which is a good approach for the therapy regime.(3) It is significant that drugs for reducing lipids, statins, can affect osteogenesis, apoptosis of osteoblasts and even coordinate with vitamin D. Periodontal patients usually have elevated levels of cholesterol and triglycerides, so they can use statins to control two non-physiological conditions in the body: periodontitis and dyslipidemia.(5)

### WNT pathway in bone remodeling

Alveolar bone must be healthy in order for the strength of the teeth to provide basic, everyday support. Bone loss is a normal phenomenon over the years and health conditions such as periodontitis can lead to tooth loss. The loss of tissue and bone around the tooth can lead to the final tooth loss(6), although this process can be prevented, patients usually seek help when the disease is already in an advanced stage(3). Interfering with stem cells during bone remodeling, the WNT pathway is important for its renewal and can be a potential drug therapy target. The influence of the canonical WNT pathway on the dynamic balance and differentiation of bone cells by means of beta-catenin at the nuclear level, makes this pathway important in bone biology.(7)

### SOST and DKK-1

Acting at the level of stem cells in coordination with  $\beta$ -catenin, which can activate the production and differentiation of osteoblasts at the genetic level, the canonical WNT pathway is extremely important in bone biology. SOST and DKK-1, glycoproteins and inhibitors of WNT canonical pathway, whose effect begins in the extracellular space by binding to Frizzled (Fz) proteins, are crucial for this review. The entire binding process is coordinated by Lrp-5/6, and the signal is transmitted via the DSH phosphoprotein to the  $\beta$ -catenin destruction complex which prevents the decay of  $\beta$ -catenin. Stabilized  $\beta$ -catenin coordinates with transcription factors in the nucleus and realizes its effect.(7) The main production site of SOST is in osteocytes as well as its expression in chondrocytes and cementocytes.(8) It is important to emphasize that the immune system leads to the inflammatory process of periodontium through the activation of leukocytes (polymorphonuclear), causing the inflammatory process to reach the bone matrix supported by microorganisms and cause its resorption.(5) Stimulation of bone resorption and significant effect on the basal part of the bone are characteristics of SOST, while DKK-1 has a significant role in reducing type II collagen production and bone resorption caused by inflammatory processes.(3) The significance of the canonical pathway is reflected in its early activation in the period between 3 and 5 days after bone fracture at the level of osteocytes, osteoblasts and osteoclasts.(7) The effect of mandibular bone matrix loss in estrogendeficient mice was reversed by DKK-1 and SOST antibodies. It was also observed that the production of DKK-1 was increased in mice lacking molars. SOST and DKK-1 antibodies significantly suppressed the effect of bone loss due to hypoocclusion and this and other studies on mice confirm the increase in alveolar bone volume when these proteins are inhibited. (9) Antibodies can increase the volume of alveolar bone, increase bone formation and decrease bone resorption, thereby preventing loss and stimulating the formation of new bone matrix. The previously mentioned can be an adequate therapeutic target for bone loss treatment.(9)

# BMP

BMP is cytokine involved in bone remodeling processes by inducing their formation.(10) The BMP pathway is more dominant compared to WNT during bone regeneration, and the therapeutic action of BMP-2 or BMP-7 is emphasized. BMP-2 is released by macrophages, the M2 type, which participates in osteoclastogenesis by clearing apoptotic cells so that bone can develop and grow again.(6) The interaction of sclerostin with BMP-2 occurs on seventh and fourteenth day, and BMP promotes ossification of  $\beta$ -catenin, while in its complete absence healing is not possible.(7) High price (up to 10,000 USD) and unexplored side effects were the main reason why BMP is currently not widely used for therapeutic purposes.(7)

# Statins

Statins are drugs that reduce cholesterol levels in the blood (5) and have antioxidant, antiinflammatory, anitimicrobial and healing effects (11). Interfering with apoptosis of osteoblast and osteogenesis, statins may reduce bone loss and improve clinical outcome with periodontal patients. Most naturally derived statins (from mushrooms) such as lovastatin, simvastatin and pravastatin are used for therapeutic purposes; as well as chemically modified such as atorvastatin, fluvastatin, cerivastatin and rosuvastatin. Different mechanisms of action; such as interfering with mevalonate (simvastatin), reducing osteoclastogenesis (atorvastatin) or activating BMP-2 (lovastatin); contribute to their additional additive significance. It is important to emphasize that simvastatin achieves a positive effect on collagen fibers by preventing their disintegration and contributing to the reduction of progression of periodontal disease.(5) Application of the drug locally to the place of response in case of oral bone damage, e.g. 1,2% rosuvastatin gel, would allow optimal concentration of the drug and maximum effect.(12) In a study in which simvastatin was applied locally for 70days, the function of osteoblasts increased by 46%, as well as another study where the same drug was prescribed in a concentration of 10mg/kg showed progress already after 8 days in reducing the inflammatory process and improving the alveolar bone structure.(5) Scientists agree that statins are the future in the treatment of bone deficits in periodontitis, but dose must be adequately adjusted according to the current state of health and there is still a lack of sufficient scientific evidence to include them as a therapeutic solution.

# Diabetes and smoking

Study conducted on diabetic animals reveals that ratio between bone remodeling and bone resorption is three times higher in favor of resorption as well as its tendency to make oral environment more in dysbiosis. Human studies prove that the oral cavity is richer in bacterial organisms and their toxins in periodontal patients with diagnosed diabetes.(6) In the study of Miranda et al. patients with diabetes had higher levels of SOST in their serum and DKK-1 in tissue compared to healthy individuals.(13)

Reduction in antioxidant expression and increased levels of ROS, as well as toxin combination of smoke from the cigarette, can be trigger for osteoclastogenesis and bone resorption. The upregulation of SOST and DKK-1 is consistent with the theory of negative WNT impact on bone formation.(6) Conclusion

Scientists have been trying to explain all the peculiarities and differences of the GCF as well as periodontitis, but the range of possibilities seems to be endless. Research that includes the interaction of SOST and DKK-1 with statins is still unknown and is important in order to define new therapeutic strategy. The simplicity of therapeutic application defined on the basis of known parameters that participate in bone breakdown and the inflammatory process of the periodontium are the future that can make life easier for the individual.

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# PERIODONTITIS AS A RISK FACTOR FOR THE DEVELOPMENT OF SYSTEMIC DISEASES

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### Abstract:

Periodontitis is a chronic infectious disease that attacks the alveolar bone and leads to inflamation and tooth loss. It is usually the result of poor oral hygiene, but stress, smoking, or certain systemic diseases can further exacerbate the condition. In addition, several studies have shown that periodontitis is a risk factor for the development of numerous systemic diseases, such as cardiovascular diseases, neurological diseases, etc. Bacteria in periodontal pockets can spread through the blood and lead to the development of atherosclerosis and subsequently to heart attack or stroke. Accordingly, timely treatment of periodontitis and oral health care play an important role in the prevention of the above diseases.

Key words: periodontitis, atherosclerosis, oral hygiene

Periodontitis is a chronic inflammatory disease that, if left untreated, can lead to irreversible damage to the periodontal tissue (gingiva, periodontal ligament, cementum and alveolar bone) and subsequent tooth loss. Periodontitis is a very common disease: approximately 50% of adults over 30 years of age and 70% of adults over 65 years of age suffer from some form of this disease. One of the most important factors in the development and progression of periodontitis is the increased concentration of pathogenic bacteria in dental plaque, which activate the immune response [1,2].

Several risk factors such as smoking, poor oral hygiene, diabetes, some medications, age, and stress are connected with periodontal disease. However, an imbalance in this system, which may be caused by a weak immune response, leads to the development of systemic disease. It starts with the formation of plaque due to the accumulation of bacteria around the tooth and the development of inflammation. Plaque is an accumulation of microorganisms that adhere to the surface of the tooth. As the biofilm matures, gramme-negative anaerobic bacteria develop. Later, the alveolar bone is destroyed and the epithelial attachment is lost. Some bacterial species connected with periodontitis are involved in the development of systemic diseases. Periodontopathogenic bacteria are classified according to their pathogenicity and characteristics into closely related complexes (Figure 1) [3,4].



Fig 1. Classification of the complex of periodontopathogenic bacteria

Over 500 species of bacteria can be isolated by subgingival and supragingival tests and classified by color. Bacteria of the vellow, green, blue, and purple complex are called early colonizers. They can attach to the pellicle. The orange complex consists of periodontal pathogenic bacteria such as Fusobacteirum nucleatum. These bacteria, associated with other periodontal bacteria form the scaffold for colonization. The red complex consist of higly pathogenic bacteria, which include: Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia are the main pathogens of periodontitis. The presence of bacteria of the red complex and Aggregatibacter actinomycetemcomitans represents the final stage of colonization. The creation of certain enzymes and toxins is associated with the pathogenesis of these bacteria. The increased concentration of molecules such as lipopolysaccharides promotes the release of inflammatory cytokines and mediators, which cause the release of matrix metalloproteinases. In this way, bone remodeling and resorption occurs. For this reason, research on the systemic effects of periodontitis on systemic health has increased. Namely, periodontal pathogens can destroy periodontal tissue, allowing harmful endotoxins and exotoxins to enter the bloodstream. As inflammation progresses, periodontal bacteria, bacterial products, immune complexes, and inflammatory mediators reach various sites in the human body. This process guides to bacterial colonization and systemic infection, which leads to activation of the inflammatory response. Thus, periodontal bacterial complexes have been detected in various tissues and organs of the cardiovascular system, cerebrovascular system and so on. Therefore, in addition to oral health problems, there is ample evidence that periodontal diseases are closely related to systemic diseases, diabetes, cardiovascular diseases, rheumatoid arthritis, Alzheimer's disease, respiratory diseases, etc. (Figure 2) [5,6].

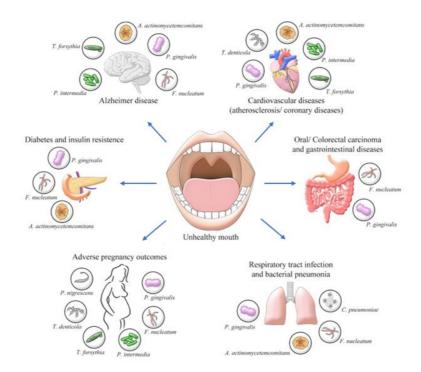


Fig 2. Association of certain periodontal pathogens with systemic diseases (modified and adapted according to [5].

Diabetes and periodontitis

Diabetes mellitus is a disease associated with hyperglycemia caused by a hereditary and/or acquired deficiency in the production and/or activation of insulin. The specific mechanism linking diabetes mellitus and periodontitis is not fully understood. Systemic levels of inflammatory mediators, which are increased in periodontal disease along with oxidative stress, may provide a link between diabetes mellitus and periodontitis. Periodontal infection and subsequent inflammation increase insulin resistance, which is due to an increase in proinflammatory cytokines and bacteremia, as discussed earlier. Periodontitis releases various inflammatory molecules that can interact with free fatty acids, lipids, and end products of glycolysis. Diabetes mellitus leads to a marked increase in fibroblast

activation and an increase in bone resorption, and all of the above factors induce osteoblast apoptosis and osteoclast formation, eventually leading to bone resorption and loss of bone volume [2].

### Periodontitis as a risk factor for the development of cardiovascular disease

Periodontal disease and cardiovascular disease are common chronic diseases whose incidence increases with age. It has been scientifically proven that there is a link between these two diseases, and periodontal disease is associated with an expanded risk of acute myocardial infarction, heart failure and stroke. There are some common factors that can deteriorate periodontitis and cardiovascular disease. The most common ones are smoking, stress, obesity, diabetes, and unhealthy diet [7].

However, the main mechanism for the association between periodontitis and cardiovascular disease appears to be the oral dissemination of pathogens into the bloodstream. For example, bacteremia, which is often caused by nonsurgical and surgical dental procedures, is a major cause of infective endocarditis. For this reason, patients with heart disease are prescribed antibiotic prophylaxis before dental procedures [8,9].

Many studies have shown that chronic inflammation is an important factor in atherosclerotic cardiovascular disease. Studies have shown that atherosclerotic lesions contain inflammatory components. The cellular interactions in atherosclerosis are similar to those in chronic inflammatory fibroproliferative diseases. A systemic inflammatory or immune response to periodontitis can lead to cardiovascular dysfunction. In addition, oral pathogens can cross the gingival barrier and enter atherosclerotic plaques via the blood, triggering an inflammatory or immune response within the atherosclerotic plaque. Circulating levels of several cytokines have been identified as biomarkers of ischemic atherosclerotic disease. There are four factors associated with periodontitis and atherosclerotic plaque formation. The first relates to the direct entry of oral bacteria into the bloodstream. The second is related to the release of inflammatory mediators that enter the bloodstream, while the third mechanism refers to an autoimmune response after exposure to an oral pathogen, and finally, the fourth mechanism describes the possibility that oral pathogens are involved in the production of toxins with proatherosclerotic effects [9,10].

In addition, periodontitis has been associated with the development of stroke. Stroke is one of the leading causes of death worldwide. Numerous recent studies have shown that the risk of cerebral ischemia and stroke is higher in individuals with periodontitis. For example, Pussinen et al. found that elevated serum antibodies to Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis were associated with stroke [8].

# Periodontal pathogens associated with rheumatoid arthritis

Close epidemiologic, serologic, and clinical associations have been demonstrated between rheumatoid arthritis and periodontal disease. Rheumatoid arthritis is an inflammatory systemic immune disease that can lead to destruction of articular cartilage and capsule and, in severe cases, to joint deformities. The presence of periodontitis may favor the progression of rheumatoid arthritis, while rheumatoid arthritis is unlikely to accelerate the development of periodontitis. There is a link between rheumatoid arthritis and periodontitis through pathological factors such as chronic inflammation caused by proinflammatory cytokines and the breakdown of connective tissue. The periodontal pathogen A. actinomycetemcomitans is considered to trigger rheumatoid arthritis and establishes a link between autoimmunity and periodontitis [2].

### Neurological disorders and periodontitis

Neurological diseases associated with changes in the brain and nervous system are among the most common diseases in the world. The connection between periodontitis and neurological diseases has recently attracted the interest of researchers. Several studies have shown that periodontitis significantly increases the risk of neurological disorders such as Alzheimer's disease. Alzheimer's disease is a neurodegenerative condition characterized by the slow and progressive loss of one or more nervous system functions, and it is also the most common cause of dementia, which has become a major societal health problem [8].

### Conclusion

Nowadays, not only patients but also clinicians and scientists need to be made aware of the need for a better understanding of periodontal and peri-implant diseases and their condition as well as their

impact on certain systemic diseases. Since the relationship is not yet fully understood, the emphasis on prevention and preventive measures remains very important. Interdisciplinary collaboration, as recommended by the World Health Organization, can have a positive impact on overall health and even prevent systemic disease. However, it is already clear that timely treatment of periodontitis and adequate oral hygiene can have a positive impact on morbidity, mortality and health care.

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## ADVANCES IN MODERN PREVENTIVE DENTISTRY

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#### Abstract:

Dental caries is a preventable infectious disease, and the early identification of risk factors and implementation of oral health preventive measures can reduce or even avoid lesion progression. Dental caries is essentially a disease caused by accumulation of acid-producing endogenous bacteria tooth surface. Bacteria live on tooth in microcolonies assembled in the form of dental biofilm (dental plaque) Some of the important changes, that have taken place over the last few years, have influenced prevention of dental caries and other oral diseases. Today we can see increased understanding of caries pathogenesis process, material and operative technique development, variety of new agents which can be used for prevention and treatment of dental caries and non-cariogenic diseases, like dentin hypersensitivity and molar-incisor hypomineralisation.

Key words: dental caries, preventive, remineralisation, dentin hypersensitivity, MIH

#### Introduction

Dental caries is a pandemic disease affecting the teeth, characterized by demineralization and cavitation, eventually leading to discomfort and pain, causing limitations in function and compromised facial aesthetics.

Dentin hypersensitivity and molar-incisor hypomineralisation are diseases with high prevalence globally. Although these conditions are frequently encountered in dental clinics, recent studies have shown that dentists experience significant difficulties in diagnosis and management of those patients.

#### Dental caries

Dental caries, commonly known as tooth decay, is a multi-factorial disease that affects millions of people worldwide. The primary cause of dental caries is the demineralization of tooth enamel by acid-producing bacteria in dental plaque. Prevention of dental caries involves various strategies that aim to control the growth of the cariogenic bacteria, to reduce the exposure of teeth to acids or to remineralize demineralized dentine.

The demineralization process involves loss of minerals at the advancing front of the lesion, at a depth below the enamel surface, with the transport of acid ions from the plaque to the advancing front and mineral ions from the advancing front toward the plaque. The remineralization process is a natural repair mechanism to restore the minerals again, in ionic forms, to the hydroxyapatite (HAP) crystal lattice. It occurs under near-neutral physiological pH conditions whereby calcium and phosphate mineral ions are re-deposited within the caries lesion from saliva and plaque fluid, resulting in the formation of newer HAP crystals, which are larger and more resistant to acid dissolution.

Numerous types of remineralizing agents and remineralizing techniques have been researched and many of them are being used clinically, with significantly predictable positive results.

Requirements of an ideal remineralization material are: to diffuse into the subsurface of enamel or to deliver calcium and phosphate into the subsurface, not deliver an excess of calcium and not to favor calculus formation, to work at an acidic pH, to work in xerostomic patients and to boost the remineralizing properties of saliva [1-3].

Remineralizing agents have been classified into the following:

Fluorides- the use of fluorides as remineralizing agent have been known for decades. Fluoride inhibits demineralization as the fluorapatite crystals, it enhances remineralization as it speeds up the growth of the new fluorapatite crystals by bringing calcium and phosphate ions together and third, it inhibits the

activity of acid producing carious bacteria. Fluoride-containing dentifrices contain fluoride in various chemical forms mainly as sodium fluoride (NaF), sodium monofluorophosphate (Na2 FPO3 ), amine fluoride (C27 H60 F2 N2 O3 ) in Elmex<sup>TM</sup> toothpastes, stannous fluoride (SnF2 ), or combinations of these. The fluoride released is absorbed to the mineral surface, as a CaF2 or a CaF2 -like deposit, in free or bound form. Stannous fluoride provides fluoride and stannous ions where the latter act as an antimicrobial agent. Toothpaste formulations containing 1426 ppm F as sodium fluoride or 1400 ppm F as amine fluoride gave a significant protection of enamel from erosive acid challenges in vitro compared to 0 ppm F placebo toothpaste. Another mode of fluoride delivery is the light-activated fluoride (LAF) treatment method, where the fluoride topical treatment is immediately followed by the application of intense monochromatic light sources, such as light emitting diodes (LED) or halogen curing lights (470–500 nm) and blue argon ion laser (488 nm).

Nonfluoride remineralizing agents

•Alpha tricalcium phosphate (TCP) and beta TCP ( $\beta$ -TCP)

- Amorphous calcium phosphate
- CPP–ACP
- Sodium calcium phosphosilicate (bioactive glass)
- Xylitol
- Dicalcium phosphate dehydrate (DCPD)
- Nanoparticles for remineralization
  - Calcium fluoride nanoparticles
  - Calcium phosphate-based nanomaterials.
  - NanoHAP particles
  - ACP nanoparticles
  - Nanobioactive glass materials
  - Polydopamine
  - PA
  - Oligopeptides
  - Theobromine
  - Arginine
  - Self-assembling peptides
- Electric field-induced remineralization

Calcium phosphate is the principal form of calcium found in bovine milk and blood. As the major components of hydroxyapatite (HA) crystals, concentrations of calcium and phosphate in saliva and plaque play a key role in influencing the tooth demineralization and remineralization processes.

 $\beta$ -TCP - the combination of TCP with fluoride can provide greater enamel remineralization and build more acid-resistant mineral relative to fluoride alone. When it is used in toothpaste formulations, a protective barrier is created around the calcium, allowing it to coexist with the fluoride ions.

Amorphous calcium phosphate (ACP) is the initial solid phase that precipitates from a highly supersaturated calcium phosphate solution and can convert readily to stable crystalline phases. It plays as a precursor to bioapatite and as a transient phase in biomineralization. The conversion of ACP to apatite at physiological pH has been described as followings: initially, there is dissolution of ACP, then reprecipitation of a transient solid phase through nucleation growth, and, finally, hydrolysis of the transient phase into the thermodynamically more stable apatite by a topotactic reaction. A biologically active restorative material containing ACP as a filler encapsulated in a polymer binder, can stimulate the repair of tooth structure by releasing significant amounts of calcium and phosphate ions. Enamelon<sup>TM</sup> is a toothpaste, consisting of unstabilized calcium and phosphate salts with sodium fluoride. It has been shown to be superior to conventional fluoride dentifrice in preventing root surface caries in radiotherapy patients.

CPP–ACP is a two-phase system which when mixed together reacts to form the ACP material that precipitates onto the tooth structure and elevates calcium levels in the plaque fluid. GC Tooth Mousse Plus<sup>TM</sup> and MI Paste Plus<sup>TM</sup> are formulations of CPP–ACP with incorporated fluoride to a level of 900 ppm, where the fluorides give additive effects in reducing caries experience. It is available as toothpastes, chewing gum, lozenges, and mouth rinses.

A bioactive material is defined as a material that stimulates a beneficial response from the body, particularly bonding to host bone tissue and to the formation of a calcium phosphate layer on a material surface. Bioglass (BG) is a class of bioactive material which is composed of calcium, sodium,

phosphate, and silicate. They are reactive when exposed to body fluids and deposit calcium phosphate on the surface of the particles. In vitro and in vivo studies have shown that BG particles can be deposited onto dentine surfaces and subsequently occlude the dentinal tubules by inducing the formation of carbonated HAP-like materials.

Nanoparticles have better ion release profiles than microparticles. Since it is difficult to directly use nanomaterials to remineralize teeth in the oral environment, these materials are often added to restorative materials as inorganic fillers, such as resin composites to release calcium, phosphate, and fluoride ions for remineralization of dental hard tissues. Nano-sized HAP (n-HAP) is similar to the apatite crystal of tooth enamel in morphology and crystal structure. So it can be substituted for the natural mineral constituent of enamel for repair biomimetically. ACP nanoparticles, as a source of calcium and phosphate ions, have been added to composite resins, ionomer cements, and adhesives. A study using in situ caries models of humans have revealed that nanoACP-containing nanocomposites prevented demineralization at the restoration–enamel margins, producing lesser enamel mineral loss compared with the control composite.

Xylitol is a tooth friendly nonfermantable sugar alcohol which has been shown to have noncariogenic as well as cariostatic effects. It exerts the anticariogenic effects by the inactivation of S. mutans and inhibition of plaque's ability to produce acids and polysaccharides. When consumed as mints or gum, it will stimulate an increased flow of alkaline and mineral-rich saliva from small salivary glands in the palate. Increased salivary flow results in increased buffering capacity against acids and high mineral content will provide the minerals to remineralize the damaged areas of enamel [4-24].

#### Dentin hypersensitivity

Dentin hypersensitivity (DH) has been defined as a short, sharp pain which arises from exposed dentin in response to non-noxious stimuli, typically thermal, evaporative, tactile, osmotic or chemical, and that cannot be ascribed to any other form of dental defects or diseases. Studies have demonstrated variations in the prevalence of dentin hypersensitivity, ranging from 1 to 98%. In patients, the affected teeth become sensitive to generally non-harmful environmental stimuli. Gentle touch, mild cold or hot, chemical (acidic or sweet fruits, foods, drinks) and air-flow stimuli can induce short sharp pain that may affect daily activities including eating, drinking, speaking and tooth brushing. It is also known that the oral health related quality of life in patients with DH is highly affected by this condition. Etiology of DH is connected with dentin exposure, especially exposure of open dentinal tubules, and dental pulp nerve responsiveness to external environmental stimuli. Dentin exposure can be caused by physical, chemical, pathological, biological challenges and/or developmental abnormalities that result in dental and/or periodontal damage or defects. Various clinical conditions like attrition, erosion, corrosion, abrasion and abfraction could induce DH. Periodontal tissue loss or gingival recession is another major predisposing factor since this leads to exposure of cervical and root dentin [25-27].

A number of theories have been proposed to explain pulpal noci-receptive transduction observed with DH. The most widely accepted mechanism for DH has been the hydrodynamic theory proposed by Brännström. It states that environmental, mechanical, thermal, and chemical changes cause the movement of fluid within dentinal tubules, which stimulate the terminals of pulpal nerve fibers located within the tubule inlet walls, thereby inducing transient acute pain. The hydrodynamic theory highlights the concept that a number of different stimuli can evoke similar responses.

A definitive diagnosis for DH is usually reached through exclusion of other conditions that need varieties of treatment options. Any condition causing dentin exposure, dental pulp hyperemia, dental nerve sensitization and neuropathy may induce short sharp pain even with only minor provocation. Clinical examination and testing are requisite for differentiating DH from other causes of hypersensitivity. Initially, the presence of exposed dentin must be identified by visual/tactile examination of the teeth. Gingival recession leading to exposure and wear of cementum is another predominant sign that should raise the level of suspicion for a diagnosis of DH. In addition to visual inspection, it is essential to evoke or induce the characteristic transient sharp pain by applying a stimulus to the affected tooth.

Strategies for management of DH include: 1) Oral hygiene education and brushing technique instruction for prevention of DH; 2) Behavioral control and elimination of predisposing factors for DH; 3) Non-invasive treatments for pain relief through occluding dentin tubules and blocking

nociceptive transduction/transmission; 4) Restoration or surgical treatments for dental hard and soft tissue defects.

Education, instruction, and engagement in prevention of erosive and abrasive tooth wear and gingival recession should be routinely provided to dental patients. the acids from vinegar, fruit and fruit juices, as well as soft drinks (e.g. citric and phosphoric acid) are the major cause for dental erosion, consumption of the acidic food or beverages should be regulated in patients prone to the development of DH.

Desensitizing toothpaste is one of the most common products used to treat dentine hypersensitivity. These toothpastes contain ingredients such as potassium nitrate, strontium chloride, or fluoride, help to block the tubules in the dentin and reduce the transmission of pain signals to the nerve. Desensitizing toothpaste is typically used twice a day and can take several weeks to achieve full effect. Application of desensitizing agents, like amine fluoride, is the most frequently used non-invasive treatment for DH. Amine fluoride is a type of fluoride that is commonly used in dentistry for its beneficial effects on oral health. It is a compound that contains a positively charged nitrogen atom, which makes it highly soluble and effective at binding to tooth enamel.

Desensitizing agents or analgesic treatments aim to suppress nerve impulses by directly stopping the nociceptive transmission occurred within dentin-odontoblasts nerve terminal complex of the dental pulp by filling in the exposed tubules in the dentin. Based on the mode of their administration, the desensitizing treatment can also be classified into at-home therapy or in office therapy categories. At home desensitizing products include toothpastes, mouthwashes and chewing gums. In-office desensitizing products can be found in the form of gels, solutions, varnishes, resin sealers, glass ionomers, and dentin adhesives. In-office desensitizing treatments also include more sophisticated laser techniques.

In the treatment and prevention of DH, no uniform evidence-based clinical guidelines are available. Prevention measures are especially important, since there is evidence that teeth already damaged by erosion are more vulnerable to acidic attacks. Studies investigating the effect of fluoride in commercial toothpastes on dental erosion confirmed the effectiveness of fluoride on dental erosion only sets in with a higher dose. Organic substances like arginine and casein phosphopeptide-stabilised amorphous calcium phosphate (CPP-ACP) show varying results in the prevention of DH [27-29].

#### Molar-incisor hypomineralisation

The term molar-incisor hypomineralisation (MIH) is a developmental, qualitative enamel defect caused by reduced mineralisation, which leads to enamel discolouration and fractures of the affected teeth. MIH typically affects the molars and incisors, in both jaws, resulting in weakened and brittle enamel and dentine, that are more susceptible to decay and other oral health problems like hypersensitivity.

Epidemiological studies from different parts of the world show a wide variation in the prevalence of MIH which can range between 2.8 to 40.2%,9 however, this variation may be due to a lack of standardised tools to record MIH leading to underestimation of the prevalence.

The etiology of MIH is complex and multifactorial, and still not fully understood, but it is believed to be a multifactorial condition with both genetic and environmental factors, during early childhood development. One of the main causes of MIH is thought to be disruption in the amelogenesis process most probably occurring in the early maturation stage or even earlier at the late secretory phase in the formation of the tooth enamel. This can occur due to a variety of factors, including:

Prenatal or perinatal factors: MIH has been linked to a variety of factors that occur during prenatal or perinatal development, such as premature birth, low birth weight, infection and exposure to environmental toxins during pregnancy.

Childhood illnesses or infections, such as measles or chickenpox, have been associated with an increased risk of MIH.

Medications: The use of certain medications during childhood, such as antibiotics or antihistamines, has been linked to an increased risk of MIH.

Nutritional deficiencies: A lack of essential vitamins and minerals, particularly calcium and Vitamin D, during childhood development can also contribute to the development of MIH.

Genetic factors: There is evidence to suggest that genetic factors may also play a role in the development of MIH. Studies have identified several genes that may be associated with the condition, although more research is needed to fully understand the genetic basis of MIH.

The ideal time to diagnose MIH is as soon as it is clinically apparent either in primary or permanent dentition. The examination should be performed on clean wet teeth. The clinical presentation of MIH depends on its severity and can range from white-creamy opacities, yellow-brown opacities, post-eruptive enamel breakdown to atypical caries located on at least one molar with or without incisor involvement. The lesions should be larger than 1 mm to be recorded as MIH. When such clinical signs exist during examination, the dentist should ask the parents about any illness that occurred in prenatal, perinatal, postnatal or the first three years of life to support the diagnosis.

According to the clinical presentation, MIH could be devided into three severity levels: mild, moderate and severe MIH. In mild MIH, the demarcated opacities are located at non-stress bearing areas, no caries is associated with the affected enamel, and there is no hypersensitivity, incisor involvement is usually mild if it is present. Moderate MIH is diagnosed when the demarcated opacities are present on molars and incisors, the post-eruptive enamel breakdown is limited to one or two surfaces without cuspal involvement. Atypical restorations can be needed and treatment for dental sensitivity. Severe MIH is condition when post-eruptive enamel breakdown and crown destruction is present. Caries is usually associated with affected enamel, severe of dental sensitivity and aesthetic concerns [30-35].

Therapy of patients with MIH is very complex. The first step will be identification of patients at risk of MIH, children with poor general health, and early diagnosis can lead to more effective and conservative management. The available treatment modalities for teeth with MIH are extensive, ranging from prevention, restoration, to extraction. The decision on which treatment should be used is complex and is dependent upon on a number of factors. The commonly identified factors are the severity of the condition, the patient's dental age and the child/parent's social background and expectation. Prevention is important and sensible to start, approaching the affected children and their parents with appropriate dietary advice. Toothpaste with a fluoride level of at least 1,000 ppm F should be recommended. Casein Phosphopepetide-Amorphus Calcium Phosphate (CPP-ACP), which provides a super saturated environment of calcium and phosphate on enamel surface, has been shown to enhance re-mineralisation. For patients with spontaneous hypersensitivity, professional application of fluoride varnish (e.g. Duraphat 22,600ppm F) and possibly 0.4% stannous fluoride gel may be helpful [36].

#### Conclusion

In recent years, the focus of restorative dentistry has been directed toward a conservative approach, out of which remineralization procedures are the most preferred and optimal way of regeneration of lost tooth structure. The preventive approach of identification, conservation, and non-restorative treatment of incipient caries, hypersensitivity and hypomineralization, saves both dental tissues and suffering for the patient.

It is expected that further experiments in this field would definitely bring out better products and technologies for clinical application with optimal responses and results.

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# FROM PERIODONTAL HEALTH TO DISEASE: THE IMPACT OF SMOKING

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#### Abstract:

Smoking causes changes in periodontal tissues, dental biofilm formation, host immune response – all affecting progression of the disease, as well as healing after periodontal treatment. It is estimated that new ways of tobacco and nicotine product consumption - electric cigarettes and Tobacco heating systems (THS), which produce aerosol with nicotine, contain less harmful products than conventional cigarette smoke, however it has not been investigated in vivo in oral cavity. Our Faculty carries out clinical studies aimed at researching of the impact of smoking and THS, on oral mucosa, teeth and periodontal tissues and to what extend smoking modifies treatment protocols.

Key words: Nicotine; Periodontitis; Smoking.

#### Introduction

More than one billion people around the world smoke - it is estimated that one out of seven people smoke cigarettes [1]. In Croatia the situation is even worse - every fourth person is a smoker, and it is estimated that every fifth death in Croatia is related to smoking.

The cigarette smoke is a mixture of over 5000 chemicals, heavy metals and other constituents. Many of those chemicals have cytotoxic, cancerogenic, mutagenic and antigenic properties. Nicotine is proven to have the majority of undesirable effects and it is responsible for the development of addiction [1,2].

Smoking is proven to be the major environmental risk factor for various diseases – cancer, cardiovascular disease, chronic obstructive pulmonary disease, but also periodontitis. It is a very important and well-validated risk factor for periodontal diseases – second only to the dental plaque [1,2]. Exposure to tobacco smoke (passive or secondhand smoking) is also a risk factor for periodontitis – odds were 1.6 times greater for passive smokers than non-smokers [2].

On the other hand, smoking cessation is a very important measure primarily followed by biofilm removal (periodontal therapy) with beneficial effects in reducing probing depths. Changes in gingival blood flow and gingival cervical fluid volume has been noticed as soon as five days after smoking cessation [3].

Smoking has a devastating effect on several aspects of periodontal health as well as periodontal diseases – it causes changes in periodontal tissues, interferes with dental biofilm formation, modifies immune and inflammatory host response, wound healing and systemic health of the individuals – affecting prevalence, severity and progression of the disease, as well as healing after periodontal treatment [2].

Effects of smoking on periodontal tissues

Nicotine from cigarette smoke has a negative effect on cells which has been demonstrated in many in vitro studies [4], and it also causes vasoconstriction of the peripheral blood vessels. That is the reason why smoking can reduce the clinical signs of gingivitis – bleeding on probing as well as gingival bleeding during patients' daily toothbrushing routine masking the early signs and symptoms of periodontal disease. Other consequences of vasoconstriction are gingival cervical fluid volume reduction and suppression of angiogenesis [2].

#### Smoking and dental biofilm formation

The effect of smoking on dental biofilm microflora and formation (especially subgingival biofilm) was controversial in early studies. Data from studies at the beginning of the century were inconsistent – some of them found no difference in biofilm microflora between smokers and non-smokers and some of them found more periodontal pathogens in smokers. These different findings can be explained by various microbiological methods that were used in these studies [1].

In recent studies difference in biofilm microflora between smokers and non-smokers was found even in patients diagnosed with Periodontal health. Greater quantities of anaerobic microbiome with more periodontal pathogens were found in smokers [5]. Smokers with Gingivitis had greater bacterial diversity and early periodontal pathogens colonization compared to non-smokers [6]. The most important difference in biofilm microflora was that smokers with Periodontitis had more Fusobacterium nucleatum than non-smokers. It is well known that F. nucleatum plays an important role in development of mature dental biofilm and the colonization of anaerobic bacteria of the socalled red complex, as well as its local immunosuppressive capability [7]. Smoking has similar effect on peri-implant biofilm – higher prevalence of periodontal pathogens was found in smokers [1].

#### Smoking and host immune response

Besides differences in biofilm microflora, smoking also compromise host immune response to periodontal pathogens (both local and systemic) shown by impaired neutrophil chemotaxis and phagocytosis, immunosuppressive effects on the macrophages, reduced IgG antibody levels, depression of antibody responses to some antigens, higher levels of proinflammatory immune mediators, etc. Nicotine can suppress vascular growth and has various effects on fibroblasts (inhibits proliferation, migration, adhesion, growth and collagen production of fibroblasts) which may impair healing after periodontal therapy [1,2,4].

#### Smoking and periodontal diseases

Smokers have more severe periodontal tissue destruction than non-smokers measured by worse clinical parameters when periodontal disease is diagnosed: grater clinical attachment loss, graeter periodontal pockets depth, greater gingival recession and more missing teeth [2,4].

Smokers are at 3.5 times greater risk of losing tooth than non-smokers [8].

Effect of smoking, as a major risk factor for periodontitis, has been well documented and acknowledged among both scientists and clinicians. Smoking was included in the current Classification of periodontal and peri-implant diseases and conditions from 2017 as a grade modifier together with type II diabetes. Grading of periodontitis provides information about biological features of the disease: history-based analysis of the rate of periodontitis progression, assessment of the risk for further progression, analysis of possible poor outcomes of treatment and the assessment of the risk that the disease or the treatment may negatively affect the general health of the patient. In order to do that, severity of bone loss is corelated with patient age: the percentage of bone loss at the worst affected tooth in the dentition is divided by the age of the patient. There are three grades – Grade A (slow rate of progression of periodontitis), Grade B (moderate rate of progression of periodontitis) and Grade C (rapid rate of progression of periodontitis).

Smoking can directly modify the grade of periodontitis – shift the grade score to a higher value: from mild to moderate or from moderate to rapid [2,9,10].

Furthermore, Clinical Guidelines for the Treatment of Periodontitis (published in 2020.) includes smoking cessation as a risk factor control which includes all health behavioral change interventions eliminating the recognized risk factor in the first step of therapy, as well as in supportive periodontal care. So, according to this relevant guideline, smoking cessation is a part of the therapy of periodontal disease [11].

#### Effect of smoking on periodontal therapy

There are many clinical studies reporting that smoking has adverse effects on different periodontal treatment outcomes [9].

After nonsurgical periodontal treatment (scaling and root planning), a smaller pocket depth reduction and less clinical attachment gain were noticed in smokers compared to non-smokers [2]. Wound healing was adversely affected by smoking and the outcome of therapy was less favorable [9].

In a recent systematic review and meta-analysis on the impact of smoking on non-surgical periodontal therapy [4], a total of 17 studies with 948 patient were included. The post-treatment reduction of probing depth, as well as the clinical attachment gain were lower in smokers then in non-smokers. Differences were statistically significant between groups, but modest from clinical point of view – 0.33 mm reduction of pocket depth and 0.20 mm in clinical attachment gain. Those differences were greater in deeper periodontal pocket at baseline ( $\geq 5$  mm).

Furthermore, studies on long term stability after nonsurgical therapy (studies on maintenance) showed that heavy smoking (more than 20 cigarettes per day) significantly contributes to the risk of periodontitis progression, greater marginal bone resorption and tooth loss. Similar findings that smoking impairs the clinical outcome were reported in studies on surgical periodontal therapy – flap surgery, mucogingival surgery and regenerative surgery. Smoking also has negative effects on dental implant therapy – reduced success rates, greater peri-implant marginal bone loss, interference with osseointegration, increased occurrence of peri-implantitis [2].

The majority of studies showed a dose-response effect of smoking on the outcomes of periodontal therapy – the greater number of cigarettes smoked per day, the more negative effect on periodontal therapy is [9].

New ways of consuming tobacco and nicotine

Nowadays, there are new and different ways of consuming tobacco and nicotine products beside conventional cigarette smoking - electric cigarettes and Tobacco heating systems (THS).

THS produce aerosol with nicotine and represent a smoke-free system since there is no tobacco burning, only heating. It is estimated that such aerosol contains less harmful products than conventional cigarette smoke, however the influence of the aerosol has not been investigated in vivo in oral cavity.

Research at the Faculty of Dental Medicine University of Rijeka, Croatia

Croatian Science Fundation (a national grant) supports research "Environmental factors and microbiological interactions in the structure of dental biofilm" at the Faculty of Dental Medicine University of Rijeka, Croatia. Several clinical studies are being carried out to assess the impact of smoking and THS on the oral mucosa, teeth and periodontal tissues, as well as to what extend smoking modifies treatment protocols.

One study focuses on the differences in patients saliva composition and changes in their taste and scent in non-smokers, smokers and THS smokers.

The other study will analyze the characteristics of subgingival dental biofilm microflora as well as clinical parameters in the same three groups.

Finally, the last study focuses on the outcome of non-surgical periodontal therapy comparing smokers and non-smokers. Besides clinical outcomes (pocket depth reduction, clinical attachment gain, etc.), dimensional changes of the gingiva as well as quality of life after therapy will be compared among the groups.

#### Conclusion

It is clear that smoking has a significant negative impact on several aspects of periodontal health as well as periodontal diseases.

New ways of tobacco and nicotine product consumption need more clinical studies to prove their less harmful effect compared to conventional cigarette smoke on periodontal tissues, periodontal diseases and periodontal therapy.

Recent studies at our Faculty aim to focus more on that effect.

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## **APPLICATION OF CELL CULTURES IN DENTAL RESEARCH**

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#### Abstract:

The tooth derived from oral ectoderm is highly mineralized organ developed through epitheliummesenchyme interactions providing significant system for elucidating the molecular mechanisms of organogenesis. The cells used in tooth and bone development may be provided in the form of an immature intact matrix-organ culture, derived from tissues such as primary cultures or they may have been produced from tumor cells enabling standardized studies. Recently, there has been rising interest in epithelial cell tissue culture, especially oral mucosa and its application utilizing in vitro cell culture in dental research. Nevertheless, the research on tooth development is limited, considering epithelial stem cells are difficult to obtain and maintain. However, induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) could be good alternative choices in understanding the functional roles in tissue engineering of teeth.

Key words: cell culture, stem cells, odontogenesis, regenerative dentistry

#### Introduction

Cell culture is a versatile laboratory method which enables growth of cells in vitro, in controlled environment and optimized conditions required for specific research. Cell culturing was developed in the early 20th century in aim to investigate molecular and cellular biology, organogenesis, tissue growth, mutagenesis, pharmaceuticals development and genetic analysis of in vitro models of numerous physiologic and pathologic conditions. It is one of the crucial tools in basic and translational medicine, clinically mostly applied for investigation of various disease mechanisms and drug toxicity [1].

Due to possibility of gene and molecular pathways manipulation, as well as advantages such as no interference of environmental and genetic components of cell cultures, these systems are highly consistent and reproducible. Cell cultures are significant for establishing in vitro models of certain diseases, drug testing and development, vaccine production, tissue transplantation and regeneration and genetic engineering. However, one of the major limitations of this method is the discrepancy between experimental and artificial in vitro models and complex in vivo environment which complicates translation of the obtained results to human or other organism [1,2].

Regarding dental research, cell culture models are mainly used to study differentiation, proliferation and other complex processes of oral tissue development in vitro, in aim to form an analogue of the naturally grown oral tissue, commonly referred as tissue engineering, for clinical purposes such as regenerative dentistry [3-5].

#### Cell lines

Two sources of cell lines are established cell banks or donor tissue-isolated cells. European Collection of Animal Cell Cultures (ECACC) and The American Type Culture Collection (ATCC) are some of the World Health Organizations' acknowledged cell culture collections. ECACC currently provide over 40,000 cell lines. Donor cells are ex vivo resected and enzymatically, chemically, or mechanically treated to achieve uniform cell line [2,6,7].

These two methods are the base of cell culture classification into three types: primary cells, immortalized cells, and self-renewing cells. Primary cells, isolated from human or animal tissue, greatly replicate their original tissue, however they have a limited life span after few cell divisions, therefore are regarded as "finite" cell lines. Immortalized cell lines, however, are naturally generated or genetically manipulated cells. These standardized cells are either tumorous cells or cells artificially manipulated to proliferate indefinitely and provide multiple sub-culturing and passaging, therefore are referred to as "continuous" cell lines. However, this advantage can also result in genotypic and phenotypic abnormalities with less resemblance to the the original tissue and poor mimicking of the in vivo environment. Self-renewing cells have particular interest in stomatology and include cells such as embryonic stem cells and induced pluripotent stem cells. These cells can differentiate into other cell types, therefore, can be highly useful for regenerative dentistry, which will be further explained.

Following the obtaining of cell lines, the cells are cultured as monolayer (monoculture of single cell type or co-culture of two or more cell types) or 3D cultures. The issue of discrepancy between cell culture models and complexity of cell environment in reality is being tackled by recently rapidly developing 3D cultures that mimic in vivo environment to much greater extent than monolayer cultures [8].

First application of cell cultures in dental medicine has been recorded in 1955. when cell culture technique was applied for evaluation of cytotoxic properties of dental materials [9]. Since then, cell culture applications in dental medicine have expanded to research of oral mucosal epithelial barriers [10], odontogenesis, dental and oral pathological conditions such as gingivitis, periodontitis and oral cancer [11], dental stem cell banking for both scientific research and clinical purposes, such as regenerative dentistry [12,13], etc.

Cell cultures are applicable in all medical fields, and in our Laboratory of Translational Medicine at the University of Osijek, Faculty of Dental Medicine and Health they have been used to mimic various pathophysiological conditions and elucidate the mechanisms of action of different treatment options [14-18], however, in vitro studies in dental medicine are still limited. In this article, we will briefly describe recently obtained knowledge and developments in utilization of cell cultures in dental medicine, particularly stem cells, their sources and clinical utilization.

#### Sources of stem cells in dentistry

As previously mentioned, stem cells have the ability to self-renew and differentiate in various cell types. Due to that, stem cells have gained interest in the field of tissue engineering therapeutic options in dentistry. Furthermore, due to their mesodermal origins in embryogenesis [19], oral tissues have been identified as a valuable source of stem cells, such as adult mesenchymal stem/stromal cells (MSCs) which further provide source for genetically reprogrammed cells, such as human induced pluripotent stem cells (hiPSC) [20].

Adult stem cells and embryonic stem cells (hESC) are naturally present in the human tissue and represent the main sources of the stem cells. Additionally, human induced pluripotent stem cells (hIPCs) are derived from somatic cells and artificially modified by genetic manipulation, drug or chemical treatment and have the ability to differentiate into the endoderm, mesoderm, and ectoderm, while majority of adult stem cells potential to differentiate is limited only to certain cell types [21,22].

Adult stem cells, commonly referred to as mesenchymal stem cells or multipotent mesenchymal stromal cells (MSCs), can be obtained from various tissues. Regarding oral tissue, numerous sources of stem cells have been identified, such bone marrow-derived MSCs (BMSCs), dental tissue-derived stem cells which include dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), stem cells from human exfoliated deciduous teeth (SHED), stem cells from the apical papilla (SCAP), etc. Another valuable MSCs source is oral mucosa, from which few different types of MSCs have been identified, such as oral epithelial progenitor/stem cells and human gingiva-derived MSCs (GMSCs). There are also other sources such as periosteum-derived stem/progenitor cells, salivary gland-derived stem cells and adipose tissue-derived stem cells (ASCs), etc. [20].

Pluripotent stem cells (hESCs and hIPCs) are totipotent with ability to differentiate in all germ layers, while adult stem cells have limited pluripotency. The major difference between these two types of cells is that hESCs are obtained by culturing cells from the blastocyst in an early stage of embryonic development, while hIPSCs can be effectively derived from oral mesenchymal cells from easily accessed tissues, as mentioned in previous paragraph. This embryonic origin is the main limitation and ethical concern regarding obtaining hEPCs. The discovery of mechanism and potential of hESCs was a key step for developing hIPCs, while hIPCs retain the properties and behavior of hESCs, they do not raise any ethical concerns. Therefore, hIPCs have great advantage over both hESCs and adult stem cells. Possibilities of hIPCs regarding the personalized patient-specific approach revolutionized the field of regenerative medicine, resulting in hIPCs being a subject of numerous scientific research and a major source of stem cells in modern medicine, including regenerative medicine [23].

#### Application of stem cells in dental medicine

Conventional approaches for treating multiple conditions in oral pathology and dental medicine include synthetic materials as a replacement for defects and whole teeth, despite that, these materials lack tissues physiological structure and function. With the development of cell culture models and research of stem cells from above mentioned sources possibilities for alternative, stem cell-based options have evolved rapidly [4,5,24].

Some of the common pathological conditions in dental medicine such as periodontal disease, caries and fractures result in tooth loss or extraction and may lead to alveolar bone resorption. Bone resorption complicates denture and dental implants treatment, therefore tissue engineering with stem cells is a promising option of treating defects of periodontal and alveolar bone tissue in prosthodontic medicine [25,26].

Stem cells-based therapeutics are a novel field, however growing number of studies suggest banking stem cells as a promising option for regeneration of various oral tissues. Nonetheless, as a result of the complexity of oral tissue structures and regenerative medicine methods, further research is required before implementation of these strategies into clinical practice [26,27].

#### Conclusion

Cell cultures are a valuable, ethically non-concerning method, widely used in the field of translational medicine. In dentistry, cell cultures provide an efficient method for teeth development research, synthetic and biomaterials toxicity testing, and recently, stem cell-based regenerative dentistry. Numerous studies have identified sources of stem cells and their potential in treatment of periodontitis, bone repair, regeneration of the pulp and new teeth development. However, these approaches are novel, stem cell biology still requires elucidation and further in vitro and in vivo studies are essential for stem cells to progress into precise and reliable method for modern regenerative medicine.

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## VISTA - VESTIBULAR APPROACH IN PERIODONTAL AND PERI-IMPLANT SOFT TISSUE REGENERATION

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#### Abstract:

In array of many therapeutic options are available for treatment of gingival recession defects and regenerations of soft tissue a vestibular incision subperiosteal tunnel access (VISTA) showed its simplicity and applicability. The current case reports introduce a relatively novel, minimally invasive approach applicable for both isolated recession defects as well as multiple defects in the maxillary anterior region. This entails making an access incision in the maxillary anterior frenum, followed by elevation of a subperiosteal tunnel. A novel method of stabilization of the gingival margins is also introduced, referred to as coronally anchored suturing, designed to maintain the coronal positioning during healing. The current report describes the technique and two clinical case documentations for treatment of Miller Class II defect and peri-implant tissue management. Although VISTA has been applied in other regions, its application is most advantageous in the esthetic zone.

*Key words:* connective tissue graft, gingival recession, mucogingival surgery, periodontal regeneration, soft tissue augmentation

#### Introduction

Gingival recession may be treated using lot of different therapeutic options with varying degrees of success, depending on the initial presentation and treatment approach. Recent systematic reviews have widely reported the coronally advanced flap in combination with a connective tissue graft (CTG) as the gold standard for soft tissue augmentation and periodontal root coverage [1,2]. The evidence on the treatment of multiple recession-type defects, particularly Miller Class III and IV defects is scarcer. The studies on sites with interproximal attachment loss have demonstrated heterogeneous results with a mean root coverage ranging from 51.5% to 98.0% [2].

Vestibular incision subperiosteal tunnel access (VISTA) may be well suited for the treatment of multiple recession type defects with presence of interproximal attachment loss [3,4] and for variety of common periodontal and peri-implant soft tissue regeneration.

The predictive value of various parameters on the outcome of gingival recession therapy has been reviewed. These parameters have been categorized into 3 groups: patient factors, tooth factors and defect/site factors. The most important risk factors presented in the cited study are smoking, presence of interproximal bone loss (gingival recession Class III and IV), thin biotype and deep initial recession (more than 4mm) [5]. In order to determine the efficacy of soft tissue augmentation the most common method used is linear measurements using a periodontal probe, which is limited by the errors associated with utilizing an instrument that measures at millimeter scale [6]. Such methodological inaccuracies could potentially affect the conclusions reached in clinical studies. Application of digital volumetric measurements for the quantitation of the outcome of root coverage has many clear advantages. Gil A. et al [7] showed that used sensitive 3-dimensional digital analysis tools to examine the correlation between initial site-specific characteristics of patients with multiple gingival recession defects and the outcome of periodontal root coverage using VISTA thnique, that initial root prominence, loss of interdental tissue (Miller Class III), molar tooth type, initial recession depth and width were negatively correlated with the outcome of periodontal root coverage achieved. Conversely, initial gingival margin thickness was associated with increased percentage of root coverage.

Biomaterials for periodontal and peri-implant soft tissue regeneration have progressively gained popularity due to their advantages compared with autogenous grafts, such as unrestricted availability, avoidance of a secondary surgical site, reduction of the surgical time, and patient's preference [8]. Indeed, the risk of developing moderate/severe postoperative swelling and pain increased at 3% and

4%, respectively, for each minute of the surgical procedure [9]. Ideally, biomaterials should be characterized by certain properties, including biocompatibility, ease in surgical site adaptation and positioning, space maintenance, clot stabilization, tissue integration, cell invasion/guidance, and promotion of cellular proliferation [10]. Scaffold-based extracellular matrix (ECM) technologies are effective in soft tissue augmentation at periodontal and peri-implant sites. Given that these materials are devoid of cells and usually cellular signaling molecules, they promote soft tissue volume, but not keratinized tissue neogenesis. Nevertheless, ECM scaffold constructs generally encourage the migration and the proliferation of fibroblasts and keratinocytes providing an excellent color match with the surrounding tissue. The reduced surgical time and morbidity compared with autogenous grafts are some of the main advantages of these materials as patient preferences indicate [11]. The recent study sought to evaluate gingival volume changes following root coverage with the vestibular incision subperiosteal tunnel access (VISTA) procedure. Pre- and postoperative surface treated with VISTA using various graft materials were digitally superimposed to quantify volumetric changes. A linear gingival thickness gain of approximately 1 mm and volumetric gain of 5.47 mm3 were achieved. A negative correlation was found between linear thickness gain and root prominence. The thickness achieved was not different with various graft materials [12].

#### VISTA clinica technique

Vestibular incision subperiosteal tunnel access was proposed and well described by Zadeh HH [3]. After administering local anesthesia through infiltration and/or block anesthesia, the exposed root surfaces is need to be treated by scaling and root planning and odontoplasty to reduce excessive root prominence in cervical areas if necessary. Odontoplasty is performed using rotary finishing burs or ultrasonics diamond-coated inserts. Ethylene diamine tetra-acetic acid gel (24% pH balanced; PrefGel, Straumann, Basel, Switzerland or some other) should be applied for 3minutes to remove the smear layer and expose collagen fibers [13] even though previous studies have failed to demonstrate additional clinical benefits nor detrimental effects of root surface chemical conditioning [2]. A vertical vestibular incision of sufficient length should be made in a suitable location to allow access to the surgical area. The typical location of this incision in the anterior maxilla is in the midline frenum. For the posterior maxilla, as well as any location in the mandible, the position of the initial incision could be between the canine and lateral incisor. The incision is made through the periosteum to elevate a subperiosteal tunnel, exposing the facial osseous plate as well as root dehiscences. This tunnel is extended at least one or two teeth beyond the teeth requiring root coverage to mobilize gingival margins and facilitate coronal repositioning. A microsurgical periosteal elevator (VISTA 1, Dowell Dental Products) who proposed by author Zadeh HH [3] is used to create the subperiosteal tunnel. The VISTA elevator is introduced through the vestibular access incision and inserted between the periosteum and bone to elevate the periosteum, creating the subperiosteal tunnel. It is important to extend the tunnel elevation sufficiently beyond the mucogingival margin as well as through the gingival sulci of the teeth being augmented to allow for low-tension coronal repositioning of the gingiva. Additionally, the subperiosteal tunnel is extended interproximally under each papilla as far as the embrasure space permits, without making any surface incisions through the papillae. Use of an elevator with bayonet curves (VISTA 2 and 3, Dowell Dental Products) facilitates access to the gingival sulcus and interproximal areas from the vestibular access. The tunnel must release sufficiently to advance the gingival margins coronal to the CEJ with minimal tension.

A simple interrupted suture or double horizontal mattress sutures (6.0 polypropylene with C3 needle) should be positioned approximately 3 mm apical to the gingival margin. The teeth need to be then etched for 10 seconds. If crown restorations were present, etching should be done for 1 minute with porcelain etchant (10% hydrofluoric acid). Each gingival margin was then repositioned at least 2 mm coronal to the CEJ of the tooth and every suture knot was bonded in position to the facial surface of the teeth with flowable composite. The clinician made the selection of an appropriate graft material, based on clinical considerations, such as the presence and thickness of the preoperative zone of keratinized gingiva, esthetic demand, number of recessions treated, and root prominence. The graft materials that could be used included autogenous connective tissue from palate or tuberosity, acellular dermal matrix allograft (Perioderm; Musculoskeletal Tissue Foundation, Edison, NJ), or xenogenic collagen matrix (XCM, Fibro-Gide; Geistlich Pharma, Wolhusen, Switzerland) in combination with or without platelet derived growth factor (PRF, PDGF). The graft material should be inserted inside the tunnel and stabilized to the overlying mucosa by placement of 6.0 polypropylene interrupted sutures. A fine-tipped curved serrated forceps may be used to insert the collagen membrane or other graft

material inside the subperiosteal tunnel. Alternatively, the membrane may be guided using a lasso suture within the tunnel by inserting a 4.0 silk suture with a 22-mm, 3/8 circle needle subperiosteally within the gingival sulcus of the distal-most tooth and exited through the midline access incision. The suture is then passed through the edge of the membrane and returned through the same path of entry to exit from the distal tooth sulcus. Once the membrane is properly positioned, the silk suture is removed and the membrane is carefully repositioned below the gingival margin of each tooth. The initial vertical incision was approximated with 5.0 chromic gut or 4.0 black silk sutures. The sutures at initial vertical incision should be removed 1 week and the others sutures fixed on vestibular surface of the teeth 3 weeks postsurgically. Patients were prescribed antibiotics (amoxicillin or clindamycin), naproxen sodium 550 mg or ibuprofen 400mg every 12 hours when needed and chlorhexidine rinse 0.12% twice a day for 3 weeks.

#### Case reports

#### Patient 1

Patient 1 was a 41-year-old woman who presented with a 2-mm Miller Class I recession defect at the left maxillary lateral incisor and left maxillary canine, with a lack of peri-implant soft tissue thickness in region of left maxillary central incisor (Fig. 1a). This was the case 4 month after placement an implant and before the second surgical phase. The procedural steps outlined in the "VISTA clinical technique" section were followed. The sutures at initial vertical incision were removed 1 week and the others sutures fixed on vestibular surface of the teeth 3-week postoperative visit. Figure 1d illustrate after 12 months of postoperative follow-up, complete root coverage of natural teeth was observed along with sustained thickness gains of keratinized gingiva in peri-implant tissue.



Fig.1a. Miller Class I recession defects were seen at the maxillary right central incisor and at the left maxillary canine, with a lack of peri-implant soft tissue thickness in region of left maxillary central incisor



Fig.1b. Using a vertical vestibular incision distally from maxillary right central incisor, a subperiosteal tunnel was created and gingival margins were repositioned coronally and anchored to teeth by bonding sutures to each tooth with composite resin



Fig.1c. Xenogenic collagen matrix (XCM, Fibro-Gide; Geistlich Pharma, Wolhusen, Switzerland) was guided through the tunnel and properly positioned to cover all facial osseous plate as well as root dehiscences overlying the root and peri-implant surfaces. The vertical incision was approximated and sutured primarily with multiple 4.0 black silk sutures.



Fig.1d. After 12 months of postoperative healing and prosthetics rehabilitation, complete root coverage and interdentally papilla was maintained along with thickness gains of keratinized gingiva in peri-implnat tissue

#### Patient 2

Patient 2 was a 49-year-old woman before prosthetic rehabilitation in upper jaw who presented with 6mm Miller Class II recession defect on maxillary right canine (Fig. 2a). Figures 2b illustrate this case 5 month after treated recession defect by the VISTA approach using as a graft material only autogenous connective tissue from tuberosity. After prosthetics rehabilitation and 12 months of follow-up, 85% root coverage was noted, along with 2-3 mm gains in keratinized gingiva (Fig. 1c).



Fig. 1a



Fig. 1b



Fig. 1c

#### Conclusion

An important technical difference between the VISTA and other approaches and more classical techniques of gingival augmentation is the degree of coronal advancement of the gingival margin advocated during the procedure. As noted earlier, the gingival margin, with it's attached to autogenous connective tissue or collagen membrane, is advanced to the most coronal level of the adjacent interproximal papillae rather than to the cementoenamel junction. Polypropylene sutures are then secured to the facial aspect of each tooth, effectively preventing apical relapse of the gingival margin during the initial stages of healing but compensating for some degree of apical migration or micromotion during the healing period. The minimally invasive VISTA approach presented in these case reports, could be combined with a broad wound-healing growth factor (PRF, GBR with beta-tricalcium phosphate hydrated with recombinant human platelet-derived growth factor BB) or with proper substitute for autogenous connective tissue (acellular dermal matrix allograft, xenogenic collagen matrix or scaffold-based extracellular matrix) affords a number of unique advantages to the successful treatment of single or multiple recession defects and peri-implant soft tissue regeneration.

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## DIODE LASERS IN DENTISTRY, DENTIN HYPERSENSITIVITY - THE BEST THERAPEUTIC APPROACH

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#### Abstract:

The aim of the research is to verify the effect of the treatment with diode laser SiroLaser Blue (660nm) of DH alone or in combination with different impregnating agents. Fifty patients in five groups were included in this research. All the patients have been asked to define the level of DH using VAS (0-10). The first group was treated with Fluor Protector, the second group Fluor Protector with diode laser, the third group was treated with Vivasens, the fourth group Vivasens and diode laser. The fifth group has just been treated with SiroLaser Blue (660nm). The efficiency of the treatment was checked immediately after the conducted treatment, after 7 days and 1 month. Vivasens and diode laser irradiation have provided the best results in the review of mean values after the first examination and one month after the treatment of DH.

Key words: Dentin hypersensitivity; Desensitizing; Diode Laser; Non-carious cervical lesion

#### Introduction

Dentine hypersensitivity (DH) is a frequent clinical problem with an increasingly higher rate of prevalence. This represents a long-term painful discomfort for the patients, and for the dentists, it represents a diagnostic and therapeutic challenge (1).

Although hypersensitivity can occur in any part of the tooth, the most common is in the cervical part on the vestibular side and the surface of the root. The frequency of this occurrence is from 3 - 57%, and in patients suffering from periodontal disease, it is more common, 72 - 98% (2).

Many theories have been trying to explain the mechanism of DH. The theory that was most widely accepted originated as a hypothesis, and was amended in 1972 (Brannstrom and Astrom) under the title "hydrodynamic theory" which tried to explain the occurrence of pain by moving fluid within the dentine tubules (3). Based on hydrodynamic theory, several methods are based on blocking open dentine tubules, such as the application of fluorides, dentine adhesives, corticosteroids, and silver nitrate (4).

In the last few decades, classic treatments with desensitizing agents are supported by lasers (5). Most of the studies conducted with different types of laser, with different wavelengths and application time, reveal the effectiveness of this treatment, immediately after the conducted treatment as well as after several months from the first treatment. As a result, the pain is reduced, and in many cases, it disappears (6, 12).

#### Objective

The aim of the research is to verify the effect of the treatment with diode laser SiroLaser Blue (Dentsply Sirona) of DH alone or in combination with different impregnating agents.

#### Material and methods

Fifty patients have been included in this research and they were randomly separated into five groups. Every patient has undergone anamnestic diagnostic protocol: general anamnesis (information about patient's general health and habits–smoking) and clinical evaluation of periodontal status (verification of Plaque index, Papilla bleeding index and Gingival index).

DH has been determined in all the patients using a visual analog scale (VAS), in a way that DH was stimulated using an air syringe of the dental unit from the distance of circa 1 cm from the tooth neck. All the patients have been asked to define the level of pain using VAS with a score of 0 to 10, where 0 represents "not in pain" and 10 "most painful". All the subjects gave their written informed consent for the treatment of DH. After verifying DH, Fluor Protector (Ivoclar Vivadent) was applied to one group

(group 1) of patients in the treatment in a way that the tooth was isolated from the saliva, dried with a cotton ball, and Fluor Protector was applied according to the manufacturer's instructions. After the application of Fluor Protector, the second group (group 2), has undergone irradiation with SiroLaser Blue laser (660nm) using the program pre-specified by the manufacturer. The third group (group 3), after the already-described tooth preparation, has had impregnating agent Vivasens (Ivoclar Vivadent) applied, and the fourth group (group 4), after the application of impregnating agent Vivasens, has had diode laser irradiation conducted using SiroLaser Blue (660nm) using the same program, already specified by the manufacturer. After verifying DH, the fifth group (group 5) has had their tooth isolated in an above-mentioned manner and irradiation was conducted using SiroLaser Blue laser (660nm), using the same program, already specified by the manufacturer.

Immediately after the conducted treatment and at the control examination after 7 days and 1 month, the efficiency of the treatment was checked using VAS for every group. All the obtained data has been noted into the work chart specifically designed for this research.

#### Results

For the statistical analysis of the received data, a program package SPSS for Windows (version 21.0, SPSS Inc., Chicago, Illinois, the USA) and Microsoft Excell (Version 11, Microsoft Corporation, Redmond, WA, the USA) were used. Nominal and ordinal variables in the research were analyzed with the  $\chi^2$  test and T-test. For the limit of statistical significance, a value of  $\alpha$ =0,05 was used.

		Sex				Total	
		Male N	%	Fema N	ale %	Ν	%
	age 18-29	3	6	22		25	50
	age 30-39	4	8	12	24	16	32
	age 40-49	2	4	5	10	7	14
	age 60-69	1	2	1	2	2	4
Total		10	20	40	80	50	100

Table 1. Sex and age structure of the subjects

χ2=2,696 (p=0,441)

A total of 50 patients participated, both male and female, within the age range of 18 to 69.

Comparison in the treatment of dentine hypersensitivity	Fluor protector	Vivasens
Before treatment	1,3	1,1
Immediately after the treatment	0,8	0,8
Seven days after the treatment	0,7	0,6
One month after the treatment	0,6	0,6
T=0,384 (p=0,195)		

Table 2. Comparison of Fluor protector and Vivasens method of dentine hypersensitivity treatment

Comparison in the treatment of dentine hypersensitivity	Fluor protector	Fluor protector plus Sirolaser Blue irradiation
Before the treatment	1,3	1,3
Immediately after the treatment	0,8	0,8
Seven days after the treatment	0,7	0,6
One month after the treatment	0,6	0,4

Table 3. Comparison of Flour protector and Flour protector plus diode laser irradiation

T=0,302 (p=0,77)

Comparison in the treatment of dentine hypersensitivity	Vivasens	Vivasens plus Sirolaser blue irradiation
Before the treatment	1,1	2,4
Immediately after the treatment	0,8	1,3
Seven days after the treatment	0,6	0,9
One month after the treatment	0,6	0,3

T=0,98 (p=0,36)

Table 5. Comparison of different means for the treatment of dentine hypersensitivity plus diode laser irradiation

Comparison in the treatment of dentine hypersensitivity	1 I I	Vivasens plus diode laser Sirolaser Blue irradiation	
Before the treatment	1,3	2,4	1,3
Immediately after the treatment	0,8	1,3	0,7
Seven days after the treatment	0,6	0,9	0,5
One month after the treatment $T_{-0,00}(n=0,020)$	0,4	0,3	0,5

T=0,09 (p=0,929)

	Dentine			Dentine
	• •			hypersensitivity one month after
	treatment	-		the treatment
		treatment	treatment	
	Mean	Mean	Mean	Mean
Flour protector	1,3	0,8	0,7	0,6
Flour protector plus diode laser Sirola Blue irradiation	aser 1,3	0,8	0,6	0,4
Vivasens	1,1	0,8	0,6	0,6
Vivasens plus diode laser Sirolaser E irradiation	31ue2,4	1,3	0,9	0,3
Diode laser Sirolaser Blue irradiation	1,3	0,7	0,5	0,5

Table 6. Comparison of different methods for the treatment of dentine hypersensitivity

T=9,181 (p=0,0001)

#### Discussion

In our research on the treatment of DH, we used desensitizing agents that are available on our market, and that are different by their chemical characteristics, as well as diode laser with the program intended for the treatment of DH (660 nm). Our results showed that all of the desensitizing agents used in the research have shown a reduction of DH immediately after the application, as well as after seven days and after one month, but there was no statistically significant difference between these two groups (table 2.), which corresponds to the conclusion made by Samuel et al. (7). Similarly, results obtained by Ravishankar et al. have shown significant pain reduction indicated using a visual analog scale (VAS) from the initial value in all three groups in all time intervals, and it also revealed that Admira Protector is better with pain reduction in DH than PRG protective coating and polyfluoride varnish after one month of application (8). The aim of the clinical research by Ozlem et al. was to determine and compare the effectiveness of the glutaraldehyde agent (GCA), Nd: YAG, Er, Cr: YSGG laser, and their combination in the treatment of dentine hypersensitivity (DH). Even though the research was conducted on only 17 subjects, the researchers have concluded that laser Er, Cr: YSGG has a promising potential in the treatment of DH (9). The conclusion of this research corresponds with our results that the use of diode laser provides better long-term treatment results. In our research, Fluor protector plus diode laser irradiation immediately after treatment did not show significantly better results. By observing the treatment method using two different techniques – namely, Fluor protector and Fluor protector plus SirLaser Blue irradiation, it can be seen that the second method provided a better result after only seven days, and after one month it had an even more significant effect. The difference has proven to be statistically significant (p>0,05) (table 3.). Also, the application of Vivasens desensitizing agent plus diode laser irradiation has proven more effective concerning the application of Vivasens alone. The treatment during which diode laser was also additionally applied has given twice as better treatment results after one month (table 4.). The results of our research have shown that the combination of desensitizing agent Vivasens with diode laser irradiation has proven the most effective (table 5.). Within the limitations of their study, Zeol LF et al. (10) find that today's discoveries indicate that, regardless of the clinical experience, dentists in Brazil still find dentine hypersensitivity a challenge for an everyday dental practice, with which we agree. In our research, the application of diode laser alone has not proven superior concerning other treatment methods that have been used in the research. Vivasens plus diode laser irradiation has provided the best results even after one month since the treatment of DH (table 6.), which corresponds to the results by Thamyres Maria Silva Simões et al. (11). Based on the obtained data we find that the combined treatment provides better treatment results concerning the use of laser alone or conventional topical desensitizing agents in the treatment of DH. Still, we find that new, better-designed, studies are needed on this topic for conclusions to be reached. The results also indicate that existing knowledge on DH should be expanded to facilitate diagnosing and planning the most effective treatment for each patient individually. Regarding the rising prevalence of DH in clinical practice, an effort should be made to educate undergraduate students on the latest findings in this area.

#### Conclusion

Based on obtained results, we can conclude that desensitizing agents (Vivasens, Fluor Protector, Ivoclar Vivadent) and the use of diode laser SiroLaser Blue (Dentsply, Sirona) alone have provided good treatment results in DH immediately after the treatment.

The treatment with diode laser SiroLaser Blue (660nm) in combination with a desensitizing agent (Vivasens) has proven to be more effective in the treatment of dentin hypersensitivity, and its effect was longer lasting.

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## PROBIOTIC SUPPLEMENTATION IN DENTAL CARIES PREVENTION

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#### Abstract:

The mechanism of probiotic activity is still a subject of many researches. Probiotic species competitively inhibit the formation of toxic substances and the growth of less desirable species by occupying their space for colonization and nutrition. Probiotic use is considered safe and significant, due to their positive effects, such as immunomodulation, hypocholesterolemic activity, protection against infections, and immune response normalization.

Most probiotics are Gram-positive bacteria that belong to the genera Lactobacillus or Bifidobacterium. Studies based on the use of the intestinal probiotics Lactobacillus rhamnosus GG, Lactobacillusreuteri, and Bifidobacteriu have each reported achieving reduced levels of Streptococcus mutans.

Increased S. mutans count is associated with a higher risk of caries and its more rapid progression. Given the essential role of S. mutans in the development of caries, efforts have been made to influence its prevalence and cariogenic ability in the oral cavity.

After usage of probiotics, there have been shown the suppressed growth of S. mutans and other oral streptococci with cariogenic potential has been demonstrated in vitro.

Based on a review of the available literature, it can be presumed that the microflora in children is less stable and more susceptible to change compared to the microbial communities in adults. Consequently, probiotics may have a more lasting effect on the resident microbial population in children. By using probiotic products, it is possible to modify dental biofilm composition and metabolism.

*Key words: lactobacillus rhamnosus GG; caries prevention; probiotics* 

#### INTRODUCTION

Inclusion of probiotics in a wide range of food products is attracting increasing interest due to their potential health benefits. The World Health Organization (WHO) defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host"[1]. Probiotic use is considered safe and significant, due to their positive effects, such as immunomodulation, hypocholesterolemic activity, protection against infections, and immune response normalization [2].

Probiotics impair or retard bacterial colonization [3], affect mucosal permeability and restitution of microorganisms in the digestive tract, and impede inflammatory processes. In the oral cavity, probiotics bind to bacteria and proteins and thus influence bacterial metabolism, as well as production of substances with an inhibitory effect on cariogenic bacteria [4].

Probiotics are typically ingested through consumption of:

• food and drink supplemented with cultures (fruit juices)

- probiotic fibers inoculated with probiotic cultures
- dairy products (milk, yogurt, kefir, cheese)
- dietary supplements (in powder, capsule, or tablet form) [5].

More recently, probiotics started to be more intensively used in dentistry, as constituents of toothpaste, chewing gum, tablets, lozenges, and mouthwashes [6].

In the oral cavity, more than 800 different types of bacteria can be identified, making it one of the most complex microbial populations in the body. Such three-dimensional bacterial communities, incorporated into the glycoprotein matrix (comprising of bacterial extracellular polysaccharides and saliva and gingival sulcus products) and attached to solid dental tissues, such as enamel or root surface, are denoted as dental biofilm [7].

For decades, it has been known that the main cariogenic bacterium Streptococcus mutans (S. mutans) is one of the dental biofilm constituents. It is the most important microorganism for the development of caries, both because of its rapid metabolism of sucrose, glucose and fructose, which lowers the pH, and due to the fact that it alters microbial homeostasis towards the caries-causing flora. Increased S. mutans count is associated with a higher risk of caries and its more rapid progression. Given the essential role of S. mutans in the development of caries, efforts have been made to influence its prevalence and cariogenic ability in the oral cavity. Authors of several clinical studies have investigated the use of probiotics, after probiotics have been shown in vitro to suppress the growth of S. mutans and other oral streptococci with cariogenic potential [8,9].

Extant studies have also shown that dental hard tissue disease is associated with an increased presence of acidogenic and acidouric bacterial strains, especially S. mutans, which has a distinct demineralizing potential. These cariogenic strains, through their metabolic activity, very quickly convert the fermentable sugars into weak organic acids, lactic acids in particular, thus exhausting the host's buffering capacity, which lowersthe dental biofilm pH. When the buffering capacity is completely exhausted and the low pH values persist for a long period, a large number of microorganisms that predominate in the dental biofilm under neutral pH conditions begin to decrease drastically, i.e., change in microbial flora occurs, marked by dominance of cariogenic bacteria [1].

Studies aimed at establishing the role of probiotics in caries prevention are largely based on the prevention of streptococcal growth, especially S. mutans [10]. In most of these cases, probiotics contained in milk, ice cream, yogurt, and other dairy products have been examined. Empirical evidence also shows that probiotic technology is a revolutionary approach to maintaining optimal oral cavity health [11].

Lactobacillus rhamnosus GG (LGG) is a probiotic with a very important role in dentistry, as it is believed to reduce caries prevalence in children [12,13] and was shown to confer the same benefits in adults when combined with fluoride [14]. Like other probiotics, LGG produces lactic acid that penetrates the bacterial membrane and inhibits the proliferation of Porphyromonas gingivalis, Streptococcus mutans, and Prevotelle intermedie. It also modifies proteins at the binding site, whereby it eliminates the agglutinin gp 340, which is essential for the binding of Streptococcus mutans and inhibits the glucosyl transferase enzyme, thus preventing glucan synthesis in the process of dental biofilm formation. LGG produces antibacterial substances that are S. mutans antagonists, while it does not ferment sucrose. Moreover, it is capable of colonizing the oral cavity and thus replacing cariogenic streptococci bound to the tooth surface because the adherent ability of Lactobacillus rhamnosus for oral tissues is greater than the adherent ability of streptococci [15].

Findings yielded by several studies indicate that LGG-containing probiotic dairy products potentially reduce caries risk in children [16].

Consequently, the aim of this study was to investigate the impact of consuming Lactobacillus rhamnosus GG-enriched yogurt on dental plaque accumulation in preschool- and school-aged children.

#### METHODS

The research reported here was conducted at the Dentistry Clinic of Vojvodina in Novi Sad, at the Department of Pediatric and Preventive Dentistry. The study included 48 preschool- and school-aged children (4-12 years old) of both sexes, who formed a control group (23 children) and an intervention group (25 children). The latter consumed 200 ml of Lactobacillus rhamnosus GG-enriched yogurt (B-Activ LGG, Dukat) daily for 14 days. Prior to commencing the study, parents of the participating children were informed of all research procedures and provided signed consent for the child's participation.

Silness-Löe plaque index and saliva pH (pH-Fix-0-14, Macherey-Nagel) was determined for all subjects on the first and last day of the study.

The Silness-Löe plaque index was measured by examining four tooth surfaces (vestibular, vestibulomesial, vestibulo-distal, and lingual) with a periodontal probe, and each of the surfaces was rated on a 0-3 scale (0 = no dental biofilm, 1 = small amount of biofilm not visible to the naked eye, 2 = greater amount of biofilm visible to the naked eye, 3 = abundance of dental biofilm). The plaque index was calculated by summing the scores pertaining to all four surfaces of all teeth and dividing by four (number of surfaces examined) and the number of teeth examined in both upper and lower jaw.

The saliva pH was determined using pH-Fix indicators, which measure pH in the range from 0 to 14.

The research was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Dentistry Clinic of Vojvodina in Novi Sad.

The data obtained were analyzed using the statistical software SPSS v. 20.0. For numerical parameters, the findings were presented in the form of arithmetic means and standard deviations. The differences between these results were examined via a paired-samples t-test, whereby p<0.05 was considered statistically significant.

## RESULTS

The experimental group that consumed yogurt enriched with Lactobacillus Rhamnosus GG (LGG yogurt) comprised 25 children, 12 (48%) of whom were boys and 13 (52%) were girls. In this group, baseline saliva pH ranged from 5 to 8, increasing to6–8 after 14-day LGG yogurt consumption. The control group comprised of 23 children (11/48%boys and 12/52% girls) of preschool and school age that did not consume LGG yogurt. Both baseline and post-intervention saliva pH values in this group were in the 5–7 range. By conducting the t-test with the p<0.05 level of statistical significance, it could be determined whether the saliva pH values after LGG yogurt consumption (AC) differed significantly from the baseline (BC), and the findings are reported in Table 1.

Table 1. Paired-samples t-test with p<0.05 significance level, intervention group(independent variable: Saliva pH)

Paired-samples t-test									
				95% Co	onfidence				
	Mean	Standard	Standard	Interval					
	Value	Deviation	Error	Lower	Upper				
				Limit	Limit				
AC-BC	-0.520	0.586	0.117	-0.762	-0.278	-4.437	24	0.000174	

In the experimental group, the BC and AC Silness-Löe plaque index values ranged from 0.113 to 2, and 0.012 to 0.458, respectively, as shown in Figure 1. It is evident from the graph that LGG yogurt consumption has led to marked plaque index reduction, and these findings are supported by the t-test results reported in Table 2.

 Table 2. Paired-samples t-test with p<0.05 significance level, intervention group (independent variable: Plaque Index)</th>

Paired-samples t-test								
	Mean Value			andard <u>95%</u> Confidence Interval				
	v ulue	The Deviation End	Lower Limit	Upper Limit				
Plaque index at baseline– Plaque index after intervention	0.547	0.417	0.083	0.375	0.719	6.557	24	0.000001

Plaque index values measured for the control group at the start (in the 0.5-2 range) and end (0.2-2) of the study period, revealing minimal changes. The t-test results reported in Table 3 confirm that the noted differences are not statistically significant at p<0.05.

Table 3. Paired-samples t-test with p<0.05 significance level, control group (independent variable: Plaque Index)

Paired-samples t-test								
				95%	Confidence			
	Mean	Standard	Standard	Interval				
	Value	Deviation	Error	Lower	Upper			
				Limit	Limit			
Plaque Index at baseline								
– Plaque Index at 14-	-0.136	0.323	0.067	-0.276	0.003	-2.026	22	0.06
day follow-up								

#### DISCUSSION

Probiotic cultures competitively inhibit the formation of toxic substances and the growth of pathogenic species by depriving them of space and food [17,18]. Extant research suggests that, although probiotics cannot replace the destroyed natural flora, as temporary colonies, they can help the body perform the same functions as the natural flora, giving it sufficient time to recover [19].

The main benefit of orally administered probiotics stems from their ability to adhere to the surface of oral cavity structures and colonization. The most commonly studied oral probiotics are lactobacilli [20]. Studies on the role of probiotics in caries prevention are based on the streptococci growth inhibition, Streptococcus mutans in particular [10].

Although probiotics are thought to have a potential anticariogenic effect, their exact mechanism of action has not yet been fully elucidated. It remains to be ascertained whether Lactobacillus rhamnosus is an anticariogen due to its ability to decrease the amount of S. mutans in the dental biofilm or due to some other mechanisms. Probiotics have been shown to impair or retard bacterial colonization [3] by selecting species during dental biofilm formation [21]. Coaggregation with S. mutans is one of the most frequently studied mechanisms potentially contributing to the posited anticariogenic effect of Lactobacillus rhamnosus [21-23].

Lactobacillus rhamnosus is of particular importance for oral biology, given that it does not ferment sucrose and is less harmful to teeth than bacteria that produce large amounts of lactic acid. Controlled studies have shown the effectiveness of L. rhamnosus in caries prevention and reduction. Although it has also been reported to reduce the effect of cariogenic S. mutans, L. rhamnosus colonization in the oral cavity is highly unlikely [24].

The aim of the present study was to demonstrate the effect of the use of L. rhamnosus-enriched yogurt on the degree of dental plaque accumulation after two weeks of consumption. The results obtained by analyzing the saliva pH and plaque index in children measured before and after consuming yogurt indicated an increase in pH and a significant decrease in dental biofilm in all children, from which a decreased presence of S. mutans could be indirectly inferred. The mean saliva pH value at baseline was 6.32, increasing to 6.84after two weeks of yogurt consumption. As expected, the amount of dental biofilm declined, as indicated by the mean plaque index of .81 and 0.26, before and after intervention, respectively.

In the majority of studies examining the effect of probiotics on the oral microflora, probiotics were consumed for up to 15 days, which is in line with the methodology adopted in the present investigation. The most commonly studied probiotics are those contained in fortified milk, ice cream, yogurt, and other dairy products. For example, Chinnappa and colleagues [25] observed a decrease in S. mutans count after week long daily consumption of ice cream and whey containing a probiotic. Similar results were obtained by Caglar et al. [26] with Bifidobacterium lactis, Glavina et al. [16] with the probiotic L. rhamnosus, Chuang and Huang [27] with L. paracasei, and Burton et al. [28] using S. salivarius M18..

Although this issue has been addressed in numerous studies [2-14,29] and their findings indicated that the Lactobacillus probiotic plays a beneficial role in caries prevention, the exact mechanism of probiotic action has not been established.

Based on a review of the available literature, it can be surmised that the microflora in children is less stable and more susceptible to change compared to the microbial communities in adults, due to which probiotics may have a more lasting effect on the resident microbial population in children [9]. Owing to this disparity, the work presented here focused specifically on preschool- and school-aged children.

Numerous strains of lactobacilli have been identified, but only a small subset of these promote caries development. Available evidence indicates that L. salivarius w24, owing to its sucrose metabolism and pH-reducing capability, could act cariogenically. On the other hand, L. rhamnosus, L. paracasei, and L. reuteri can have a safe and positive effect on caries inhibition. Nonetheless, in vitro results preclude specific conclusions and recommendations. It can be generally stated that the effect of lactobacilli may be desirable in the case of carefully selected probiotic candidates [29]. It is important to emphasize that even strains of the same species have different characteristics and each should be individually investigated. It is possible that the same species is not optimal for all oral conditions; hence, bacteriotherapy should be tailored to the oral health status of each individual.

#### CONCLUSION

Regular consumption of LGG-enriched yogurt (Lactobacillus rhamnosus GG) has an inhibitory effect on the accumulation of dental biofilm and promotes saliva pH increase, and thus indirectly affects the S. mutans levels. By using probiotic products, it is possible to modify dental biofilm composition and metabolism. Since microflora in children is more susceptible to change, it would be advantageous to include products such as LGG-enriched yogurt in the regular diet of children of preschool and school age as a means of caries prevention.

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## **PREVENTION OF DENTAL CARIES IN CHILDREN**

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#### Abstract:

Dental caries is a global health problem and is considered the most common infectious disease in adults that we are exposed to during our lifetime. The current approach to caries prevention includes assessment of individual caries risk, clinical examination, diagnostics and non- or minimally invasive treatment. Various cariostatic agents are used that are even more effective than previously used methods. Silver diamine fluoride, chlorhexidine, xylitol, casein phosphopeptide and amorphous calcium phosphate and probiotics are some of the cariostatic agents that are effective in preventing caries. Preventive measures such as educating parents and children about the importance of good oral hygiene are equally important for oral health.

Key words: dental caries, prevention, cariostatic agents

#### Introduction

Dental caries is a chronic, multifactorial disease that affects more than 3.5 billion people worldwide and impairs their quality of life by destroying tooth structure (1). Recent statistical data on the incidence of dental caries in European countries show that more than 61% of children aged 6 to 12 years have caries on one or more tooth surfaces (1). Dental caries is triggered by the development of a biofilm and can lead to the destruction of mineralized tooth structure (2). The process of caries development is a series of interactions between bacteria that produce acid through further fermentation of dietary carbohydrates stored on the tooth surface in the form of plaque (3). The accumulation of bacteria in dental plaque and the production of acids leads to a decrease in the pH of the oral cavity, which over a long period of time can lead to demineralization of the tooth structure. Although the processes of demineralization and remineralization are everyday natural processes, the repeated process of demineralization over a long period of time can lead to permanent destruction of tooth structure and the development of carious lesions (2). The oral cavity harbours more than 700 different species of bacteria, viruses and fungi, including bacteria associated with the development of periodontitis and probiotic bacteria responsible for maintaining the health of the oral cavity (2). Dental caries in children occurs on the occlusal surfaces of teeth in more than 80% of cases, most commonly on the first molars (4).

Caries prevention should be based on the suppression of the most common factors for the development of carious lesions. The risk of developing a carious lesion includes various biological, physical, as well as environmental and psychological factors. The most common factors, such as inadequate diet and oral hygiene, have the greatest influence on the development of carious lesions in children (3,5).

Preventive methods try to reduce the impact of caries on the tooth structure by timely and correct diagnosis and direct the started demineralization process towards remineralization of the tooth structure (6). Dental caries can be prevented by regular and proper oral hygiene, dental check-ups, and a consistent, regular diet (7).

Topical fluoridation is one of the most widely used and very effective methods of preventing dental caries in children. The application of fluoride in the form of gels, varnishes, toothpastes and materials for restoring the structure of the teeth acts on the process of demineralization of the enamel, preventing its further progression and at the same time stimulating the remineralization of the damaged tooth surface, making it resistant to the acid environment and the action of bacterial enzymes (8). The risk of occurrence of carious lesions can be reduced by daily and regular application of low doses of fluoride (9). In addition to the use of fluoride, cariostatic agents such as casein phosphopeptide and

amorphous calcium phosphate, xylitol, silver diamine fluoride and chlorhexidine are increasingly used in daily dental practise.

#### Casein phosphopeptide and amorphous calcium phosphate (CPP-ACP)

Casein phosphopeptide and amorphous calcium phosphate form a nanocomplex obtained as a milk derivative (10). They have a strong anticariogenic effect thanks to the action of casein phosphoproteins and calcium phosphate, which increase the availability of calcium and phosphate on the tooth surface, while inhibiting the demineralization process on the surface of the tooth structure (7). The advantage of using CPP-ACP is the possibility of preserving tooth structure. It is often marketed in combination with fluorides and forms CPP-stabilised amorphous calcium fluoride phosphate, which causes an increase in fluoride concentration in dental plaque and stimulates the remineralization process (11).

#### Silver diamine fluoride (SDF)

Silver diamine fluoride is an alkaline solution containing 24.4% to 28.8% silver, 5.0% to 5.9% fluorine, and 8% ammonia that has been used since 1969 to stop the progression of caries in children (12,13). Silver and fluorine compounds have the ability to inhibit the formation of cariogenic biofilms. Fluorine in the composition of SDF binds to the cellular components of bacteria and interferes with enzymes related to carbohydrate metabolism, while silver can cause cell death of microorganisms or negatively affect the metabolic processes of microorganisms, depending on the concentration (4). Silver ions kill microorganisms by blocking respiratory enzymes without negatively affecting human cells. Silver ions can also lead to the condensation of DNA molecules in the microorganism and cause the loss of their replication ability (14). In their study, Mei et al. (2017) found that SDF reacts with calcium and phosphate ions in saliva and causes the formation of fluorohydoxyapatite, leading to the cessation of carious lesion formation (15). There are five potential mechanisms of action of SDF on dental caries: obturation of dental tubules, formation of a reaction between SDF and mineral components of the tooth leading to prevention of caries lesion formation as well as hardening of dental tissues, antienzyme action of reaction products, inhibition of matrix metalloproteinases, and aging of the mentioned fluorohydoxyapatite (13,14). The remineralized tooth surface treated with SDF preparations can be further improved by the use of synthetic biomimetic peptides, resulting in a surface that can be restored with conventional adhesive dental composites (13). The advantages of using SDF preparations are the positive effect on the caries lesions formed that would lead to the development of pain and discomfort, halting the further demineralization process, the affordable price of the product, and the ease of application of the preparation (16). Negative consequences of SDF use include discoloration of demineralized tooth surfaces, potentially negative effects on pulp tissue if the remaining portion of dentin is extremely thin compared to the pulp chamber, and effects on soft tissue and gingiva in the case of subgingival lesions (17). In addition, there is the potential toxicity of a high fluoride concentration in SDF, as well as the photosensitivity of SDF preparations (16).

Despite the numerous advantages of using SDF in pediatric and preventive dentistry, the acceptance of SDF is not very high worldwide, which is probably due to the lack of education of dentists about the benefits of using SDF as a preventive measure (13).

#### Probiotics

The oral cavity is the habitat of numerous microorganisms, and the diversity of the microbiota depends on the type of surfaces colonized, the availability of nutrients, the oral hygiene habits of the individual, and environmental factors (18). Probiotics can be described as living microorganisms that are also found in the flora of the oral cavity, where they form a natural defense against harmful bacteria and improve health when ingested in sufficient quantities in the body. The influence of probiotics can be seen in their effect on the microbial composition of the gastrointestinal tract, which directly affects the formation of dental plaque, as well as in the increase in the patient's immune response to cariogenic microorganisms and in the change in the pH of the oral cavity, which creates the conditions for a healthier environment (18,19).

Most probiotics consist of Gram-positive bacteria of the genus Lactobacillus or Bifidobacterium. Probiotics available on the market today are mostly found in the form of lozenges, chewing gum, and dairy products such as milk, yogurt, and ice cream (20). They are considered dietary supplements and may contain a single species of microorganism or a combination of several different species (21). The development of probiotic interventions to improve oral health requires knowledge of the oral microbiota and the mechanisms of its effects on oral health and disease (20). Children are susceptible

to dental caries from an early age, and exposure to probiotic bacteria could still have a major impact on oral health in adulthood (21). According to a review of literature, the most commonly used probiotics in research on the effects on caries prevention in children include Lactobacillus and Streptococcus species (18). Krupa et al. (2022) concluded in their study on the effects of xylitol-based chewing gum and rinse water with added probiotics that probiotics may be more effective in preventing caries than the above preparations without probiotics (22). In addition to the mechanical removal of plaque by the inclusion of probiotics, chewing gum with added xylitol preventively reduces the amount of cariogenic bacteria, which is crucial in patients with higher caries risk (23). The mechanism of action of probiotic bacteria on dental plaque is described as follows: probiotic bacteria participate in the binding of microorganisms to proteins, competently act on the plaque formation system and have a chemical inhibitory effect on oral bacteria (23). Numerous studies indicate that yogurt is an excellent vehicle for the use of probiotics in the daily diet (20, 21, 23).

To achieve an adequate effect of probiotics on the bacterial flora of the oral cavity, daily intake over an extended period of time is required (19,23).

#### Conclusion

Prevention is an important and indispensable step to improve the quality of oral health and overall health of children and adults. With the development of dentistry, the availability of various prevention methods has never been greater, but an indispensable part is the education of parents and children in good oral hygiene. Nowadays, there are increasingly available various cariostatic agents such as silver diamine fluoride, CPP-ACP, xylitol and probiotics, which, in addition to their antimicrobial properties, have the ability to positively influence the remineralization process by increasing the pH of the environment and modulating the developing biofilm. The choice of prevention methods, as well as the management of the patient, is an individual decision that depends on the condition of the child's oral cavity, his eating habits and the social environment in which he grows up.

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# **BIOMECHANICAL ASPECTS OF IMPLANT PROSTHODONTICS**

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#### Abstract:

Long-term success of prosthetic treatment in implantology is possible only if biomechanical principles are followed. The lecture will explain the action of forces on implants and restorations, parameters that affect their biomechanical behaviour and the mechanism of complications. Occlusal forces affect the type of forces that are transmitted to the surrounding bone and their distribution. The recommended type of occlusion is implant-protected occlusion, which is a modification of conventional occlusion concepts designed to reduce the occlusal load on implant-supported dentures. The proprioceptive mechanism of the implant (osseoperception) is less precise than in the teeth, so there is a danger of occlusal overload of the implant and consequent microtrauma. Reducing mechanical complications also involves minimizing the three clinical arms of the torque: occlusal crown height, cantilever length, and occlusal width.

Key words: biomechanics, implant-protected occlusion, torque.

#### Introduction

Biomechanics explains the laws of statics and dynamics in the world of living beings, which enables the understanding of the normal functions of organisms, predictability of changes and proposing the necessary interventions. Following biomechanical principles in prosthetic dentistry can ensure long-term success of therapy. However, the question is are there some differences between conventional and implant prosthodontics from biomechanics point of view? Roughly, the most important three are: necessity of passive fit, differences in occlusion and differences in treatment plan which is directed basically with biomechanical behaviour of implants[1]. When forces act on implant dentures, implants show biomechanical behaviour depending on different parameters. Knowing these parameters is crucial for the prosthetic therapy planning. Parameters affecting biomechanical behaviour of implants are those relating to bone and implant appearance (such as bone quality surrounding implant, implant material and implant macro design) and ones relating to prosthodontics part such as type of denture and occlusion type[1,2].

Successful implant rehabilitation will be possible if the integrity of implant-bone junction is maintained. There are three types of forces that can act on implants in the oral environment: pressure (compression), tension and shear. Bone is the most resistant to pressure, 30% less resistant to tension, and 65% less resistant to shear. In other words, compressive forces tend to maintain the integrity of the implant-bone connection, while tensile and shearing forces tend to disrupt this connection. Shear forces are the most destructive to implants and bone compared to other types of loading. Therefore, planning and designing implant denture should be aimed at establishing compression forces and reducing shear to the smallest possible extent[2].

Most denture characteristics affects the type of forces and transmission to the bone around implants. When treating partially edentulous jaw with implants, most frequent solutions are crowns or bridges, cemented or screw retained. Unlike that, for complete edentulous patients, there are two most used options: implant-retained tissue-supported removable overdentures (IRTS) and implant-retained implant-supported fixed prostheses (IRIS). The IRIS fixed denture is very common and a well-accepted treatment for the physiologic rehabilitation of completely edentulous patients. The IRIS fixed denture reintroduce internal loading of the alveolar bone, while the IRTS overdenture does not load the edentulous alveolus, because the implant components are designed to only engage and prevent the "lifting off" of the overdenture from the edentulous ridge. The decision between IRTS and IRIS

include complex planning, but the most important factors to be considered are: number and distribution of implants, type of denture or teeth in antagonistic jaw, interjaw relations and occlusion type[3,4].

Patients with IRIS dentures report higher levels of satisfaction in terms of aesthetics, phonetics, and functionality compared to patients with conventional or IRTS removable dentures. However, clinicians should be more careful in indicating IRIS dentures, precisely because of the direct load on the alveolar bone, and should take into account all parameters that affect the amount of that load: the number and distribution of implants (surface positioning of implants is more favourable comparing to linear), anteroposterior distance between implants, cantilever length, the implant/abutment internal connection type. In addition, passive fit is necessary to provide for favourable forces transmission. Type of denture retention is also important whether it is cementation or screw retained; also, denture material type and design and occlusion/guidance type.

#### Occlusion in implant prosthodontics

Occlusion is the key factor for the biomechanical behaviour of and implant denture. Occlusion affect type of forces which are transferred to the bone and the way of their distribution. When excess forces exist, tooth or implant can be affected, and the consequence for the tooth is occlusal trauma and for the implant occlusal overloading. Mechanical complications such as screw loosening and/or fracture, prosthesis fracture, and implant fracture can be caused by occlusal overload, eventually leading to compromised implant longevity [5].

The differences between tooth and implant reaction under load are fundamental due to different junction to bone periodontal ligament and functional ankylosis). Also, threshold of tactile sensation is much higher in implant comparing to tooth, and mobility in bone varies greatly in terms of movement magnitude (for tooth is 25-100 $\mu$ m and for the implant is only 3-5  $\mu$ m) and phases of motility (for tooth two phases and for the implant one phase which is linear and elastic). Therefore, every connection, especially rigid one between tooth and implant may lead to damage in peri implant or periodontal tissue. In addition, there is also difference in stress distribution. While accepting and transferring force, tooth, periodontal tissue and bone absorb and distribute load, implant cannot distribute forces favourably, and the stress concentrate in crestal bone. Also, symptoms and signs of overload cannot be seen easily in implants. Because of that, in addition to other mentioned factors, consequences of overload in implants can lead to serious mechanical and biological failures like fractures of abutments or rarely even implants[2,4,5].

Mechanism of implant overload greatly reline on the absence of control over the regulation of mandibular movements and management of the intensity of occlusal forces. Thanks to periodontal mechanoreceptors, teeth can signal whether it can handle occlusal load and adapt. That tactile control doesn't exist in implants, with mechanoreceptors providing a shock-absorbing function[4]. Therefore, too strong or premature occlusal contacts on the implant denture can cause series of microtraumas. The peri-implant tissues could be more susceptible to crestal bone loss by applying force; this can be indicated when the crestal bone around dental implants acts as a fulcrum point for lever action when a force (bending moment) is applied.

Occlusion in implant dentures should be based on the basic occlusion concepts but with some differences (table 1.). That newly introduced occlusion concept is called implant - protected occlusion. It represent modification of conventional concepts design to decrease occlusal load on implant denture. It has reported that biomechanically controlled occlusion can achieve the clinical success and longevity of dental implants. Specific biomechanical recommendations for implant - protected occlusion are:

occlusal contacts and laterotrusion guidance with buccal cusps of molars should be avoided - it frequently cause overload and unfavourable force direction;

occlusal scheme should be adapted to the jaw with less stabile reconstruction type - such as balanced occlusion in removable dentures if any;

Apply primary splinting with dentures when possible - it can transfer the load well and reduce moment loads[5,6,7].

Clinical situation	Occlusion type recommendation
Semicircular fixed implant denture (IRIS)	Bilaterally balanced occlusion when complete denture is in the antagonistic jaw. Group teeth guidance or mutually protected occlusion when natural teeth are in the antagonistic jaw. Without occlusal contact on the cantilever on the working and balanced side (cantilever in infra occlusion 100µm). Occlusion concept freedom in centre (1–1.5mm).
Removable implant denture (IRTS)	Bilaterally balanced occlusion with lingualization in central position. In heavily resorbed bone, minimally cusp inclination.
Fixed implant denture in posterior segments	Front teeth guidance. Canine guidance or group guidance if the canine is parodontally compromised. Centralised occlusal contacts, narrow occlusal field, low cusps, minimal cantilever
Single fixed restoration (crown)	Front teeth guidance. Canine guidance or group guidance Light occlusal contact in maximal bite force, without contact in initial chewing cycle Centralised occlusal contacts (1–1.5mm flat contact surfaces). Strong and secure proximal contacts.

Table 1. Recommendation for occlusion in particular cases from clinical practice.

Some researches showed that implant protected occlusion generate lesser harmful oblique implant, crown, bone and overall stresses compared to cuspally loaded occlusion [6]. Also, simulation of load investigated in fixed mandibular denture model showed that the canine guide generated more favourable tensions compared to the bilateral balanced occlusion which generated great tensions in the entire metallic framework [7].

Load moments in implant prosthodontics

As stated before, wrong planning and ignoring important biomehcanical factors can lead to biomechanical complications. Especially complex are load moments which occur when the force acts at a distance from the implant. A moment of force is defined as a vector - defined by magnitude and direction. The magnitude of the force is multiplied by the vertical distance (moment arm). This load moment is also called torque or torsional load and can be destructive to implant systems. Three most important clinical moment arms are: occlusal height, occlusal width and cantilever length. Minimization of each of these moment arms is necessary to prevent component fractures, crestal bone loss, or complete implant therapy failure[1,8].

When bone resorption exist and there is need to establish occlusal contacts to antagonistic tooth, extraoral part may be longer (occlusal height), which can cause excessive forces. In the cases of single crown, there won't be excessive forces if occlusal contacts are regular, with dominant longitudinal stress distribution. However, in complex fixed or hybrid dentures, problems can occur, and possible solutions can be in choosing wider implants, more implants or in planning denture design in order to decrease occlusal height, cantilever length and the least favourable solution is to make removable denture[1,2].

Cantilevers as a clinical moment arms can develop great moment loads from the vertical component of the force. Restorations with multiple abutments with distal cantilevers produce very complex load on the denture but also on the bone/implant juncture. The loading of restorations with a cantilever carries a high risk of developing harmful horizontal and oblique forces, especially in cases of linear implant

placement. The longer the segment of the cantilever, the greater the intensity of the load and the greater the stress concentration on the cortical bone around the neck of the last distal implant. On the other hand, implants placed in a curved line (surface positioned), present a better ability to accept loads with an acceptable dimension of the cantilever. The dimension of the cantilever in the lower jaw with surface-placed implants is up to 2.5 times the length of the distance between the anterior and posterior implants (AP). In the upper jaw, it is recommended to be 1.5 times the AP distance. From a biomechanical point of view, cantilevers longer than 7mm induce danger of overloading and stress concentration at the implant-bone interface. Fixed implant dentures in the lateral region with a cantilever can also cause harmful horizontal and oblique forces that can cause overloading of the distal implant and stress concentration in the surrounding bone. The load that is transferred to the implants in such cases is twice as high as in the case of individual restorations on the implants, while the load per implant is 1.2 times higher when making a dental bridge[1].

Occlusal width can also produce clinical moment arm. Wide occlusal surfaces increase vestibulo-oral moment arm in any occlusal load, which cause increase of compression, tensile and shear stress in bone surrounding implant. Recommendation is to decrease occlusal width for 30-40%, and to consider occlusal width to be proportional to implant diameter (figure 1.). Specifically, in implant dentures in maxilla, occlusal contacts on lingual cusps cause increased moment arms, so the recommendation is to decrease buco-lingual width on expense of lingual cusps, which are anyway out of aesthetic zone. Contacts established in that way can distribute forces along implant axial axis. In mandible, it is recommended to modificate buccal cusps in order to decrease moment arms, and lingual cusps remain the same in order to prevent tongue biting. In addition to reducing the width of the occlusal field, it is necessary to be aware of the height and inclination of the cusps, because higher and steeper cusps induce horizontal forces that have an adverse effect on the implant and the surrounding bone (figure 2.). Research has shown that every 10° increase in the inclination of the cusps, increases the shear and tension stress by 30%, to which the implant and the surrounding bone have a reduced resistance. As a conclusion about the appearance, height and inclination of the cusps - cusps on implant dentures should be as shallow and wide as possible in order to prevent mechanical complications[2,7].

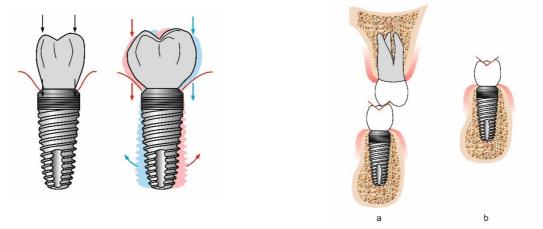


Figure 1. Occusal width as clinical moment arm. Figure 2. Cusp inclination as clinical moment arm.

#### Conclusion

In order to prevent complications in implant prosthodontics, clinician should know the implant behaviour under load, the influence of occlusion and the denture appearance on the load. Possible prosthodontics factors that can cause mechanical failures are poorly planned occlusal surfaces (wide occlusal surface, high inclination of the cusps), too intense and premature occlusal contacts and oversized cantilever.

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# **REVIEW OF MODERN ROOT CANAL FILLING MATERIALS**

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#### Abstract:

Endodontic treatment of teeth with damaged crown and pulp-dentin complex aims to prevent further damage and compromise of the apical periodontium. To prevent re-infection and achieve a high rate of success, the disinfected and instrumented root canal must be sealed three-dimensionally with root canal filling material. In modern dental medicine, the tendency is to use materials characterized by high bioactivity and biocompatibility. Materials that exhibit these properties and are frequently used in root canal treatment are Biodentine, bioceramics and mineral trioxide aggregate (MTA). In addition to the above advantages, they stimulate the formation of new dentin and the repair of defects in the hard tissue of the tooth.

Key words: Review, Root canal filling material, Biodentine, Bioceramics, Mineral Trioxide Aggregate

#### Introduction

Various clinical situations such as trauma, deep caries, iatrogenic damage or extensive tooth wear are some of the indications for pulp tissue removal. In situations where pulp tissue removal is indicated, root canal filling materials are needed to fill the empty space in the pulp cavity [1]. Instrumentation of the canals expands them and creates space for adequate canal irrigation, disinfection, and obturation. Obturation is the final step, and its purpose is to completely seal the endodontic space and prevent reinfection of canals that have been manually or engine-driven cleaned and irrigated [2]. The ideal root canal filling material has many requirements, and it is difficult to meet all of them [3]. According to Grossman [4], root canal filling materials must have the following properties, among others: easy application in the canal, biocompatibility, complete seal apically and laterally, no contraction after hardening, bacteriostatic or bactericidal activity, etc.

Nowadays, most dental practices use a combination of semi-solid obturation material - gutta-percha and cement. Gutta-percha is a natural thermoplastic material derived from the Sapotaceae tree and was first used in dental medicine in the 19th century as a temporary filling material. After the discovery of its longevity, plasticity and ability to adapt to the root canals, gutta-percha was no longer used as a temporary filling but as a root canal filling material [2]. Gutta-percha exists in two crystalline forms called "alpha" and "beta" phase. When extracted from trees, gutta-percha is present in the alpha phase, which is used in thermoplastic obturation techniques. Gutta-percha points are present in the beta phase and heating changes their crystalline form to the alpha phase [5]. Combining points and cement, creates a filling that comes closest to the ideal properties.

Modern dental medicine focuses on the use of biocompatible root canal filling materials. Since the material is in contact with periapex and vital tissue at the apical and lateral foramina, biocompatibility is one of the most important properties [6]. Biocompatibility is defined as achieving certain positive host outcome in specific applications without causing harm. The material possessing this property must not be toxic, cause adverse reactions, be irritating, cause allergies or be carcinogenic [7]. All root canal sealers have certain toxicity, especially when freshly mixed and applied. However, toxicity decreases as the sealer sets and more time passes [6]. Modern sealers should not only be biocompatible but also bioactive. In an ideal environment, the sealer should stimulate root repair and

regeneration while maintaining the vitality and structure of the tooth. Bioactive materials are Mineral Trioxide Aggregate (MTA), Biodentine and bioceramics.

#### Mineral trioxide aggregate (MTA)

Mineral trioxide aggregate (MTA) is a material made of Portland cement to which bismuth oxide has been added to increase its radiopacity. First reported use of Portland cement in dental purposes was in 1878. when Dr Witte from Germany published an article on the use of Portland cement as a root canal sealer. Almost 120 years later, Dr. Torabinejad and his colleague Dean White from Loma Linda University in California jointly developed an endodontic material based on Portland cement, which became known worldwide as Mineral Trioxide Aggregate (MTA) [8,9]. Although similar in structure, MTA contains fewer toxic metal compounds and has a smaller mean particle size than Portland cement [10]. It was first introduced in dental medicine in 1993. and approved by the FDA in 1998.

The first version of MTA was dark grey and was commercially available as ProRoot MTA (Dentsply, Tulsa) in 1998. Four years later, a "tooth-colored" version of MTA was formulated and patented [9]. Dark grey MTA had a limited clinical application. It was used in patients where the dry working area was difficult to maintain, e.g., retrograde root canal fillings. As research progressed and new technologies were invented, MTA was used for apexification, direct pulp capping and as a root canal sealer. Research has shown that white MTA, made from white Portland cement, contains less iron, magnesium and aluminum than grey MTA [11]. White MTA consists mainly of bismuth oxide and tricalcium silicate, while grey MTA contains larger amounts of dicalcium silicate and less of tricalcium silicate. Modern MTA cements contain 50 - 80% Portland cement. The chemical composition consists of dicalcium silicate, tricalcium silicate, tricalcium sulphate dihydrate, bismuth oxide and tetracalcium aluminoferrite, as well as traces of silicon dioxide, calcium oxide, magnesium oxide and calcium and sodium sulphate [12].

MTA is available as a two-component system as powder and liquid or as automix syringe. The powder mixed with an aqueous solution hardens in about three to four hours and forms a rigid structure of silicon oxide, calcium oxide and bismuth oxide. MTA is biologically and histologically similar to calcium hydroxide, has a high pH and possesses antimicrobial properties against S. aureus, P. aeruginosa and E. faecalis [13]. To optimize the physical and chemical properties of the material, research has shown that MTA must harden in the presence of moisture [14].

Concurrent research indicates that MTA is a biocompatible material to which precursor cells can adhere and form new soft or hard tissue. MTA stimulates fibroblasts to form fibrous connective tissue on which the periodontal ligament grows rapidly due to its healing capacity [15]. It stimulates immune cells to release lymphokines and growth factors which induce reparation and regeneration of cementum as well as remineralization of bone defects [16].

Due to its numerous positive characteristics and low toxicity, MTA is material of choice for direct and indirect pulp capping, pulpotomy, apexogenesis, apexification, regeneration, retrograde root canal filling and reparation of root perforation [17].

#### Biodentine

Biodentine is a relatively new restorative material developed in 2009. (Septodont, Saint Maur des Fosses, France). It is based on calcium silicate and is advertised as a "bioactive dentin substitute". Biodentine comes in capsules containing powder and aqueous liquid. Primary component is tricalcium silicate, calcium carbonate, dicalcium silicate and zirconium dioxide. Tricalcium silicate enables the setting reaction, zirconium dioxide increases radiopacity and calcium carbonate gives it mechanical properties [18,19].

Mixing the powder and solution creates a gel structure, while over time the polymerization process binds the material and forms a solid network. After mixing, calcium silicate from Biodentine reacts with water in liquid that forms high pH blend. The curing time of Biodentine is estimated to be about 45 minutes. As the material cures, the volume decreases in the first few hours due to shrinkage caused by chemical contraction, followed by secondary expansion caused by the hydration process [18]. Hydration of tricalcium silicate creates a calcium silicate gel which precipitates on surface of cement particles and gradually leads to saturation and crystal formation. Biodentine releases silicon and calcium ions over time, which induce mineralization along the dentin-cement juncture [20].

The main advantage of using Biodentine over MTA is the shorter setting time (14 minutes compared to 3-4 hours) and the higher viscosity, which allows easier handling [21]. Laurent et al. [22] demonstrated on a human tooth ex vivo that cell differentiation and mineralization was similar between Biodentine, MTA and calcium hydroxide. Biodentine and dentin have similar compressive strength, while Biodentine has a slightly higher compressive strength than MTA. Camilleri et al. [23] reported that Biodentine is denser and less porous than MTA. In a study testing sealing and apical leakage between MTA and Biodentine, MTA showed greater leakage in the first few hours, but this was controversial due to the longer setting time [24]. Root canal irrigants have no effect on the pushout bond strength of Biodentine. Furthermore, a study on extracted mandibular molars showed that blood contamination has no effect on the physical properties of Biodentine [18]. This material promotes the differentiation of odontoblasts and formation of the tertiary dentin [25].

Studies have shown that the use of Biodentine in pediatric dentistry, restorative dentistry and endodontics has produced positive results. Material is mainly used as a dentine substitute. In addition, it can be used as a provisional filling material for six months, for indirect and direct pulp capping, perforations of the pulp cavity and root canal, apexification, pulpotomy, retrograde root canal filling and internal and external root resorption [26]. Unlike other root canal filling materials, Biodentine has the disadvantage of not being radiopaque [27].

#### Bioceramics

Root canal sealants based on bioceramics (Figure 1.) have been commercially available in medicine and dental medicine for thirty years. They are a biocompatible ceramic material that includes bioactive glass, zirconia, alumina, hydroxyapatite, calcium phosphates and glass ceramics. Bioceramics are non-toxic, biocompatible, biodegradable, bioactive and dimensionally stable and chemically stable within the biological ecosystem. Due to their interaction with the surrounding tissue, they are classified as bioinert and bioactive. Bioactive glass and calcium phosphate are bioactive compounds that act on the surrounding tissue to stimulate its growth and proliferation. Zirconia and alumina are bioinert and have no biological effect on the surrounding tissue [28]. In medicine, bioceramics are used in orthopedic procedures such as joint replacements and to coat implants to increase their biocompatibility. In the treatment of dental patients, they were first used as a retrograde filling material in the 1990s. Today, they are material of choice for root repairs, sealing root canals and coating gutta-percha points [29,30].



Figure 1. Endosequence BC Sealer [31].

The mechanism of binding of bioceramics to the dentin of the root canal is still unknown, although some mechanisms have been proposed. Zhang and Li [32] suggested that the particles of the filling material diffuse into the dentin tubules and create interconnective bonds. Athem and Chong [33] proposed a different justification. In their study, they showed that the mineral content of the ceramic infiltrates into the intertubular dentin, resulting in the formation of a mineral infiltration zone after the collagen fibers have been denatured by the highly alkaline filling material.

Bioceramic materials have a high compressive strength (50 - 70 MPa) and exhibit desirable mechanical properties. Due to their high pH, which is advantageous for root canal sealants, they have an antibacterial and antifungal effect 24 hours after application in the root canal [34]. In a study conducted by Nagas et al. [35] bioceramic root canal filling material showed higher bond strength to root canal dentin than MTA.

Bioceramic-based materials are available in factory-prepared homogeneous form in syringes with cannulas which simplifies the procedure for the dentist. They can be applied straight from the packaging and inserted directly into the root canal. The exposure time is about four hours and depends on the moisture in the dentinal tubules. They are used for direct pulp capping, retrograde and orthograde root canal fillings, sealing of root canal perforations, internal and external root resorption and apexification [36]. When using bioceramics, the use of gutta-percha impregnated with bioceramics is recommended to increase the formation of a monoblock between dentin and cement and cement and gutta-percha point. Due to its good bond to the dentin, the main disadvantage of bioceramics is that they are difficult to remove once they have cured for retreatment or post-space preparation [37].

#### Conclusion

The bioactive materials MTA, Biodentin and bioceramics have hydrophilic properties and the presence of moisture is required for their curing. They are used in endodontics for orthograde and retrograde root canal fillings, repair of dentin defects, repair of fractures of root canals, direct and indirect pulp capping and external and internal root resorptions. In paediatric dentistry, they are used for apexification, apexogenesis and revascularisation. The use of bioactive materials has transformed surgical and non-surgical endodontic treatments and opens new horizons for the preservation of patients' teeth. The advantages of using bioactive materials are their biocompatibility and low toxicity, the regeneration of hard and soft tissues and the stimulation of the formation of new tertiary dentin while maintaining the vitality of the surrounding tissues. Most of these materials have rapidly gained popularity in modern dentistry due to their mechanical, physical, chemical and biological properties. However, no material has yet been developed that meets all the optimal requirements for root canal sealer. It is expected that as technologies evolve, bioactive materials will improve and be modified to overcome their disadvantages.

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# SCREENING FOR LOSSES IN INTRINSIC CAPACITY OF OLDER PEOPLE IN THE DENTAL PRACTICE

#### Kossioni A. E., DDS, PGCeODL, MSC, PhD

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#### Abstract:

The global older population is rapidly increasing due to increased longevity and low fertility. To address increased morbidity and care dependency in older age, the WHO recommends the Healthy Ageing approach that is a process of developing and maintaining the functional ability that enables well-being in old age. One of the major components of Healthy Ageing is intrinsic capacity that comprises all the physical and mental abilities of an individual that should be enhanced and maintained throughout the lifespan.

All health care providers should apply appropriate approaches in the community to detect, treat, monitor, delay or reverse declines in intrinsic capacity in order to promote well-being in old age. The dental professionals can screen for losses in intrinsic capacity all their middle-aged and older patients. Priority conditions include cognitive decline, limited mobility, malnutrition, visual and hearing impairment, and depressive symptoms. These conditions have also a close association with oral health and can be screened with simple validated tests during patient interview and clinical examination. The WHO recommends using the WHO Integrated Care for Older People (ICOPE) screening tool and referring the patient for further assessment if needed. Screening for losses in intrinsic capacity is essential in providing integrated care for older people and the dental professionals can play an important role as valuable members of the primary health care team.

# COMPUTER ASSISTED OCCLUSAL ANALYSIS IN IMPLANT DENTISTRY

#### Todorović A.

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#### Abstract:

In implant dentistry, it is believed that creating the stable occlusal scheme, with proper force loading on implant-supported restorations, could significantly impact the long-term success of restorations and implants. The use of the computer assisted occlusal analysis is one of the contemporary tools applied in order to determine localization, distribution, timing, and quality of occlusal contacts in bilateral immediate loading of implants in the posterior mandible. This lecture will cover occlusal analysis types and principles and focus on the application of computer assisted occlusal analysis in monitoring of the occlusal contacts in immediately loaded dental implants.

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# HOW TO PERFORM CORONECTOMY OF WISDOM TEETH? DIAGNOSTIC AND THERAPEUTIC TIPS AND TRICKS

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#### Abstract:

Coronectomy, as an alternative treatment option of lower impacted third molars, becomes even more accepted and popular. Its aim is to significantly reduce the incidence of irreversible inferior alveolar nerve injuries. The lecture clarifies the indications, give practical recommendations for the diagnostic presurgical workup, and shows detailed technical steps of this procedure. Experimental research-based evidence is also very briefly shown, how bone and tooth preparation instruments and their parameters should be selected.

# CYTOKINES AS MOLECULAR PAIN BIOMARKERS IN PATIENTS WITH TEMPOROMANDIBULAR DISORDER

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#### Abstract:

Temporomandibular disorder (TMD) is a multifactorial disease and the most common pain in the orofacial region affecting the masticatory muscles, the temporomandibular joint, and/or surrounding tissues with a prevalence of 5% to 12% of the population. The etiopathogenesis is complex and not fully understood, making diagnoses and treatments extremely difficult. In recent years, there have been various studies investigating the role of proinflammatory cytokines in the development and maintenance of pain and progression of TMD. In this article, we discuss cytokines as diagnostic biomarkers and therapeutic targets in TMD.

Key words: biomarkers, cytokines, temporomandibular disorder

# CLINICAL ORIENTATION OF THE OCCLUSAL PLANE USING A MODIFICATION OF THE BALKWILL ANGLE

# <u>Seifert D.<sup>1</sup></u>, Lešić N.<sup>1</sup>, Tomas M.<sup>1</sup>, Juzbašić M.<sup>1</sup>, Dragičević D.<sup>1</sup>, Horvat Aleksijević L.<sup>1</sup>, Petrović D.<sup>1</sup>, Pul L.<sup>1</sup>, Vidović N.<sup>1</sup>

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#### Abstract:

There is no unique point of view which method is most reliable and most suitable during its use. The purpose was to investigate relation between natural occlusal plane and occlusal planes determined with certain cephalometric construction of modified Balkwill's angle. The sample consists of 131 cephalograms with at least 20 natural teeth. The results shows that the natural occlusal plane differs slightly from cephalometrically determined planes, except variable Xi4. Modification Xi3 – determination of the occlusal plane by using square which upper and lower line are parallel with axis-IS+47 line, is most suitable for clinical use, because of lowest difference between natural occlusal plane, lowest measures of variability, and possible connecting occlusal plane determination and articulator's technique.

Key words: Occlusal plane, Balkwill angle

# **FULL PAPERS**

# ADVANCED TECHNIQUE FOR SMILE DESIGN IN PROSTHODONTICS AND AESTHETIC DENTISTRY

#### Djurovic Koprivica D.<sup>1</sup>, Jeremic Knezevic M.<sup>1</sup>, Milekic B.<sup>1,2</sup>, Maletin A.<sup>1</sup>, Puskar M.<sup>1</sup>, Puskar N.<sup>1</sup>

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#### Abstract:

Digital Smile Design (DSD) is the latest technical tool in aesthetic dentistry that allows each patient to create an ideal solution for their smile together with the dentist. Step by step, functionality, needs and wishes of the patient are combined with the best aesthetic solution. In this process, a series of mathematical measurements of the symmetry of the face are performed and it is very important that it is in accordance with the proportions, personality and age. DSD is a software which is based on specific photographs and software analysis and helps in easy communication between dentists, dental laboratory technician and patients.

Key words: aesthetic dentistry, smile design, digital dental software, digital prosthodontics

#### Introduction

Aesthetic dentistry doctors and specialists of prosthetic dentistry are responsible for both functional and aesthetic rehabilitation of each individual patient. Every smile is special and its design should be approached with great care. That is why it is very important that every restoration is in accordance with the proportions and shape of the face, personality and age. Harmony with psychological characteristics is essential, but also harmony with the emotional aspect of each individual.

Recently, aesthetic dentistry and dental prosthetics have undergone enormous changes in terms of technical innovations and the use of computer systems as auxiliary tools in the rehabilitation of patients. Computer-aided design and computer-aided manufacturing (CAD/CAM) technology is one of the most promising innovations in the field of prosthetics and dental aesthetics, which allows clinicians to guarantee repeatable and remarkable results to their patients from an anatomical and functional point of view [1, 2].

Digital Smile Design (DSD) is, also, advanced computerized technique represented by a modern instrument that, with the help of computer technology, allows the patient to simulate the final result of the dental treatment together with the dentist and help create the ideal solution for their smile.

The goal of the concept is to improve the aesthetic planning and design of the smile, improve communication between experts involved in the treatment, communication with the patient and increasing the interaction of the patient himself in the process of creating his own design, as well as education [3]. DSD allows dental clinicians to show the patient 2D and 3D images of his jaws and then to display to the patient how his teeth would look in each of the treatment options offered. Also, during the procedure itself, the patient can visualize what and why is being done at a given moment, so that he is better informed when he needs to make additional decisions. Experience has shown that it is much easier for patients to decide on more complex procedures if they are shown the advantages of more advanced solutions through DSD, and thanks to those devices they can see why a more complex procedure is better for them, both for the durability and quality of the solution they will choose, as well as for the visual effect [1, 2, 3, 4]. This method makes the entire relationship between the dentist and the patient transparent, because the dentist guarantees the patient that at the end of the treatment the teeth will look exactly as shown in the picture in the planning phase, whether it is natural teeth or implants. Of course, there can always be small deviations due to complications that cannot be predicted at the beginning, but if the patient insists on not deviating from the agreed solution, the doctor can always change the course of treatment so that the patient gets exactly what he wants. This is also the reason why this system is called digital smile design - because the patient visualizes almost entirely by himself how his smile will look at the end of the procedure, and the doctor is there to tell and show what is possible and what not. That's why only dentists who are completely Aconfident in

their skills use DSD, because it takes a first-class dentist to perform each procedure exactly in the way predicted by the program. Unlike traditional restorative treatments that focus on the teeth, gums and jaw, the DSD system also considers the relationship between the mouth and other parts of the face involved in smiling, such as the cheeks, eyes and lips. It also visualizes a wide range of facial expressions, to show the patient the look of his future smile when talking, laughing and in other emotional states, helping the team to design a smile that matches every individual patient personality [3, 4].

#### Development of smile design

In the last 20 years smile designing has progressively developed from traditional physical picture to digital designing which has evolved from two-dimensional (2D) to three-dimensional (3D) and even four-dimensional (4D). Nowadays it implies complete digital drawing with special computer software which is adaptable and has the possibility of changes at any moment in accordance with the patient's requirements. Team members are able to notice any discrepancies in soft or hard tissue morphology and decide which are the best available solutions using the amplified images. DSD allows for a detailed workflow simulating the complete rehabilitation of a patient, beginning with appropriately calibrated photos. The facial analyses are usually done using the reference lines, from which the standardized parameters have been developed for the frontal and profile views of the face. The horizontal reference lines used in the frontal view include the interpupillary and inter-commissural lines, which provide an overall sense of harmony and horizontal perspective, and gives a face a pleasant appearance [1, 4, 5, 6].

The best presentation of the complete evolution of digital smile design in dental prosthetics and aesthetic dentistry was presented by Christian Coachman in 2017 in six generations (Figure 1.).

Generation 1. Analogue drawings over photos and no connection to the analogue model. That was the time before introducing digital dentistry which included simply drawing on printed photos to see the future results without any link to the study model.

Generation 2. Digital 2D drawings and visual connection to the analogue model. Evolving in case of introducing Power Point software with the ability for digital drawing which brought much greater precision and less time-consuming comparing to hand drawing. Analog model link was only visual type without physical connection.

Generation 3. Digital 2D drawings and analogue connection to the model. This was the pioneer technique for digital-analogue connection which enabled the link of 2D digital smile design to 3D wax-up that very much improved this method, but 3D techniques were still not introduced.

Generation 4. Digital 2D drawings and digital connection to the 3D model. In this generation there was and huge progression of digital dentistry techniques and tools from 2D to 3D analysis which implied being able to form 3D digital wax-up using facial and marked dental aesthetic parameters. Generation 5. Complete 3D workflow.

Generation 6. The 4D concept. The most advanced technology which engages adding motion to the smile design process [2, 6].

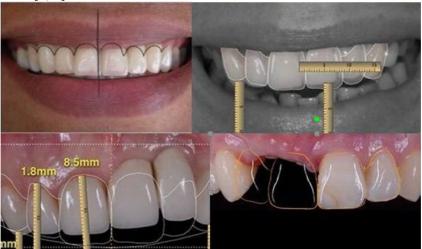


Fig. 1. Development of Digital Smile Design (taken from: https://media.digitalsmiledesign.com/christian-coachman-thoughts/smiledesignevolution.)

#### Procedure of Digital Smile Design

The whole process involves the analysis of the patients facial and dental proportions, using a predetermined series of high-quality digital photographs and videos understanding the relationship between teeth, gums, lips, smile with the facial features in motion and with emotion. No matter that in DSD professionals are using a software algorithm for predicting and designing a perfect smile, clinical smile reconstruction and designing requires a multidisciplinary dental approach that includes branches of dentistry such as orthodontics, orthognathic surgery, periodontology, and sometimes also a plastic surgery [5, 8]. For performing this procedure, there are many software available that are very similar in terms of capabilities and wealth of equipment such as Photoshop (Adobe), Microsoft PowerPoint (Microsoft Office, Microsoft), Smile Designer Pro (SDP) (Tasty Tech Ltd), Aesthetic Digital Smile Design (ADSD - Dr. Valerio Bini), DSD App by Coachman (DSD App LLC), Keynote (iWork, Apple, Cupertino, California, USA), Nemo DSD (3D) and Exocad Dental CAD, a digital SLR camera [5].

The DSD technique procedure is performed with digital equipment that is already becoming commonplace in today's dental practice, such as a computer with one of mentioned DSD software, a digital SLR camera or even a smartphone. Also, a digital intraoral scanner for a digital impression, a 3D printer and a CAD /CAM are additional tools for a complete digital 3D workflow. A true, precise and accurate photographic documentation is most important as complete facial and dental analysis rests on preliminary photos on which changes and designing are creating, a video documentation is required for dynamic analysis of teeth, gingiva, lips and face during smiling, laughing and speaking in order to integrate every guided principle to design of the future smile [3, 4, 5, 8].

A digital impression of both the jaws is taken with a digital intraoral scanner. The impressions are then uploaded to the CAD/CAM processing machine where they are 3D-printed. Three basic photographic views are necessary: full face with a wide smile and the teeth apart, full face at rest, and retracted view of the full maxillary arch with teeth apart [5, 6, 8].

A digital photography and digital analysis protocol enables the dentist to visualize and analyze issues that they may not be able to notice clinically. Drawing of reference lines and shapes over extra- and intraoral digital photographs can easily be performed using presentation software. The DSD allows for precise evaluation of the results obtained in every treatment phase. The sequence of treatment is organized on the slides with photographs, videos, notes, graphics, and drawings. At any time, team members can access the slide presentation to track and analyze the treatment provided. With the digital ruler, drawings, and reference lines, easy comparisons can be made between pre- and posttreatment photographs. These comparisons help determine whether the treatment has successfully followed the original plan or if other adjunctive procedures are necessary to improve the final outcome. Static photographs taken at a particular time cannot guarantee the ideal moment captured at the idealistic rest position and a real maximum full smile position, so because of those videos, which can be paused and transformed into a photo by making a screenshot of the best recorded moment at the desired angle, are very helpful digital tool in this procedure. The main goal of the DSD technique using special computer software is to input and adjust the photos from the 3 main views of DSD (12 o'clock, frontal and occlusal) with each other, controlled by the digital ruler, to add the lines and drawings which will help and with the help of video analysis to create the Smile Frame (Figure 2.) [1-5, 8, 9].

Digital workflow proceeds as follow: the cross (two lines placed on the center of the slide, forming a cross); digital facebow (determining the horizontal reference face line); smile analyses (evaluation of the relationship of the facial lines with the smile); smile simulation (according to the incisal edge position, canting, shifting, tooth proportions, and soft tissue outline); transferring the cross to the intraoral images (analyzing the intraoral photographs in accordance with the facial references); measuring tooth proportion (comparing the width and length proportion of the central incisors with the ideal ones); tooth outline (selection of tooth shape depending on factors such as the morphopsychologic interview and the patient's desires, facial features, and esthetic expectations); white and pink esthetic evaluation (relationship between the soft tissues and teeth, papillae heights, gingival margin levels, incisal edge design, and tooth axis); digital ruler calibration (transferring the length of one of central incisor to the computer); transferring the cross to the cast (measurements transferring to the cast with the aid of a caliper) and fabrication of diagnostic wax-up, mock-up and try in faze [3, 4, 7-10].

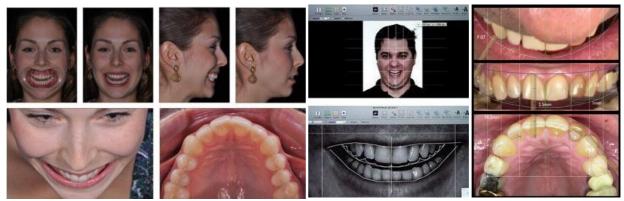


Fig. 2. Digital workflow of Digital Smile Design (taken from: https://media.digitalsmiledesign.com/christian-coachman-thoughts/smiledesignevolution.)

Conclusion

Digital smile design is more than an excellent innovative technical solution that enables a better and more successful therapeutic approach to various clinical cases, especially in those indications that refer to the aesthetic zone and require paying special attention to the facial expression and appearance of the patient himself. Cooperation with the patient, spending less time during preparation, visualization and the possibility of predicting the final aesthetic results are special advantages of this modern technology.

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# **ABSTRACTS**

### **ODONTODISPLASYA - CASE REPORT**

### Kasa Ž.<sup>1</sup>, Supić M.<sup>2</sup>, Jovanov M.<sup>3</sup>, Vujkov S.<sup>4</sup>, Komšić J.<sup>4</sup>, Nešković I.<sup>4</sup>

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#### Abstract:

#### INTRODUCTION

Odontodysplasia (ghost teeth) is rare idiopathic structural irregularity where complete odontogenesis is disturbed, that is, teeth development is stopped. Anomaly can occur in both dentitions, but most often affects several teeth of one quadrant of the upper jaw, namely central, lateral incisors, canines, and much less often premolars and molars. Affected teeth most often remain in the jaw or erupt late. Odontodysplasia occurs extremely rarely, and is more common in girls. Although odontodysplasia is idiopathic, latent viral infection, local trauma, ischemia, radiation, nutritional and metabolic disorders in the body, disorders of the migration of ectomesenchymal cells from the neural crest, and local vascular defects its development. Clinically, it is observed that the teeth in one quadrant do not appear, and the child complains of painful sensations in the area where the teeth are missing. Odontodysplastic teeth most often do not sprout, but an abscess, enlargement and inflammation of the gingiva are formed. If the teeth appear in the mouth, their color is yellow, the crowns are small, rough with hypoplastic and hypomineralized changes, and the roots are short and irregular. An X-ray reveals a very unusual appearance with a huge pulp chamber, a thin layer of enamel and dentin and barely visible and shortened roots.

#### CASE REPORT

A nine-year old girl comes to the clinic because of missing teeth 41, 42, 43, 44 and 45. An intraoral dental examination reveals that teeth 41, 42, 43, 44, and 45 are missing. Hetero-anamnesis provides the information that the milk teeth in the mentioned region were also of irregular structure, also negating diseases of importance for heredity and genetic disposition in the family history. The analysis of the OPT image revealed the presence of slight shadow embryos of the mentioned teeth. At the follow-up examination in two months, the patient denies subjective complaints and a control OPT scan was taken, where a stronger shadow of tooth 42 is observed. In further therapy, a multidisciplinary approach and consultations with specialists in jaw orthopedics and dental prosthetics will be indicated in order to provide adequate functioning and facilitate everyday life as functionally and aesthetically an accordance with the patient, s age.

#### CONCLUSION

Odontodysplasia is a rare anomaly in children, but it is a big challenge in pediatric dentistry.

# PAROTID GLAND SWELLING AFTER CHLORHEXIDINE MOUTHRINSE: CASE REPORT

### Dejanović K.<sup>1</sup>, Gušić I.<sup>2,3</sup>, Stojilković M.<sup>2</sup>, Veljović T.<sup>2</sup>, Mirnić J.<sup>2</sup>, Milekić B.<sup>2,3</sup>, Đurić M.<sup>2,3</sup>

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#### Abstract:

Enlargement of the parotid salivary gland is an extremely rare side effect of chlorhexidine (CHX) mouth rinse. The pathogenic mechanism of this phenomenon has not been determined but it is assumed that vigorous rinsing may cause the mouthwash to enter the duct and cause inflammatory reaction and transient obstruction. The aim of this case report is to highlight the parotid gland swelling as a possible side effect of chlorhexidine mouthwash. A healthy 43-year-old male underwent scaling and root planning of molars without local anesthesia. After finishing treatment he was instructed to rinse with 0.2% chlorhexidine mouthwash two times a day for two weeks. After six days, patient presented at Department of Periodontology and Oral Medicine, Clinic for Dentistry of Vojvodina, reporting sudden swelling of the left cheek and difficulty opening the mouth. Clinical examination verified the diffuse edema in the left parotid region with discrete redness of the skin but no fever or pain on palpation. An intraoral examination and panoramic radiograph excluded acute odontogenic infection and other oral pathological conditions. It was assumed that the swelling occurred as a side effect of the CHX mouthwash and the patient was advised to stop rinsing. The swelling started to decrease on the first day and after seven days a complete regression of symptoms was observed. In the differential diagnosis of sudden parotid gland enlargement, the adverse effect of CHX mouthwash should be considered in order to apply an optimal therapeutic approach.

Key words: Chlorhexidine, Parotid gland swelling, Mouthwash

# THERAPY OF PSEUDOPROGENIA IN MIXED DENTITION

### Kukolj I.<sup>1</sup>, Ivanović T.<sup>2</sup>, Ragaj M.<sup>3</sup>, Ivanović D.<sup>4</sup>, Nedić K.<sup>5</sup>

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## Abstract:

INTRODUCTION

Progenia is an orthodontic irregularity that occurs due to the discrepancy between the lower and upper jaw in the sagittal direction.

*OBJECTIVE* – description of the therapeutic procedure in a patient with pseudoprogeny in mixed dentition.

*INTRAORAL FINDINGS - underdevelopment of the maxilla, high palate, all incisors in an inverted fold, crossbite due to transverse underdevelopment of the maxilla. The lower dental arch has a regular appearance.* 

*EXTRAORAL FINDINGS* - concave profile, the upper lip is behind the N-vertical, the lower lip and chin are correctly placed in BP.

ANALYSIS OF STUDY MODELS - underdeveloped maxilla, normally developed mandible.

ANALYSIS OF TELEREDGEN - reduction in the value of the maxilla corpus, mandible corpus within normal limits Retrognathism of the maxilla and normognathism of the mandible.

THERAPY OF PSEUDOPROGENIA IN MIXED DENTITION is reduced to stimulating the development of the maxilla using active orthodontic appliances of different designs.

Key Words: progeny, retrognathism, normognathism

## DISC DISLOCATIONS OF THE TEMPOROMANDIBULAR JOINT

# <u>Bojinovic M.<sup>1</sup></u>, Djurovic Koprivica D.<sup>1</sup>, Maletin A.<sup>1</sup>, Milekic B.<sup>1,2</sup>, Puskar M.<sup>1</sup>, Puskar N.<sup>1</sup>, Jeremic Knezevic M.<sup>1</sup>

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#### Abstract:

INTRODUCTION: Disc dislocations or internal derangements of the temporomandibular joint are both mechanical disorder and abnormal position of the articular disc regarding condyle of mandible or articular eminence. During disc dislocation, discal ligaments are elongated and translatory movement of the condyle discal complex occur. Also, hyperactivity of the lateral pterygoid muscle will lead further deterioration of following structures.

MATERIAL: The most often displacement and the first stage of disc disclocation is anterior disc disclocation with reduction. Symptoms and signs are: sound of clicking during opening, normal range of mouth opening, mandibular deviation and pain is not present. If not treated, disclocation with reduction could lead to disclocation without reduction, which is progressive form. Symptoms and signs of disc disclocation without reduction are: restricted mouth opening, deflection, pain, click as well as crepitus.

Diagnostic of disc dislocation comprimes special prepared diagnostic protocol and specific imaging modality. The most useful and contemporary diagnostic protocol is "Diagnostic Criteria for TMD", according to Schiffman et al (2014). The best imaging modality for evaluation and visualisation of shape and position of the articular disc is magnetic resonance imaging.

The initial therapy of disc diclocation implies reversible occlusal therapy. Revesible therapy are making of occlusal splints, using non steroid anti-inflamator drugs, physical therapy and psychotherapy.

CONCLUSION: According to investigations, the prevalence of disc disclocation in general population is 35-42%. Considering the large number of patients in the population with disc displacement, it is necessary to pay more attention to the diagnosis of these disorders during the dental examination.

# THE CORRECT INDICATION – BASIC PRECONDITION IN DIASTEMA THERAPY

# <u>Milekić B.<sup>1,2</sup></u>, Jovanović N.<sup>2</sup>, Stojiljković N.<sup>2</sup>, Jeremić-Knežević M.<sup>2</sup>, Maletin A.<sup>2</sup>, Đurović-Koprivica D.<sup>2</sup>, Puškar N.<sup>2</sup>, Mijatov I.<sup>2,3</sup>

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#### Abstract:

Introduction

Aesthetic and functional solutions to problems related to diastema in dental prosthetics are the subject of many controversies until now. The principals of modern prosthetic rehabilitation permit us a large selection of solutions, but the most important thing is properly setting the indication and the patient's requirements regarding his future replacements.

#### Materials and methods

A 35-year-old patient was presented with the desire to partially close the space between the first upper incisors. Planning of future fillings was performed on studio models and by photographic processing. The replacements, ceramic veneers, were made of lithium disilicate ceramic using the CAD-CAM method. The cementation was performed with Variolink Esthetic LC cement.

#### Conclusion

Technical and technological advances allow us to achieve maximum replacements that are both aesthetically pleasing and functionally acceptable, always respecting the individual characteristics of the patient.

Key words diastema, ceramic veneers, aesthetics

# DYNAMICS OF FLUORIDE ION RELEASE FROM TWO CONVENTIONAL GLAS-IONOMER CEMENTS: A COMPARATIVE STUDY

#### Cvjetićanin M.<sup>1</sup>, Ramić B.<sup>1</sup>, Drobac M.<sup>1</sup>, Vukoje K.<sup>1</sup>, Kantardžić I.<sup>1</sup>

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#### Abstract:

The aim of this study was to comparatively present the dynamics of fluoride ion release from two conventional glass-ionomer cements. Material and Methods: FUJI IX (GC, Tokyo, Japan) and Ketac Molar (3M/ESPE, St. Paul, MN, USA) were used in the research. Ten discs with a diameter of 10mm and a thickness of 2mm were prepared for each material (n=5) according to the manufacturer's protocol. The samples were stored at 37°C and 100% humidity for 1 hour to allow the material to set. The samples were then immersed in 10ml of a solution of distilled water and TISAB III buffer in a 10:1 ratio. Analysis was performed using an ion chromatography test. The amount of released fluorides was recorded after 24h, 72h, 7 and 14 days, and statistical analysis was performed using a one-way ANOVA test, with a significance level of p < 0.05. Results: Total fluoride release was higher for FUJI  $(13.35\pm3.2mg/l)$  compared to Ketac  $(9.1\pm3.1mg/l)$  but without statistical significance. The release of fluoride in both materials was most intense in the first 24 hours, after which there was a lower growth of ion release, with almost stagnation reached on the 14th day for both materials. FUJI showed higher fluoride release than Ketac at all time points, but without statistical significance. The difference in fluoride release between first, third and seventh day on one hand and the 14th day on the other hand, for both materials, was statistically significant, while there was no statistical significance between the other time points. Conclusion: Both tested materials showed the most intense release of ions in the first 24 hours, while the intensity of ion release decreased over time. FUJI showed a higher degree of ion release than KETAC at all time points.

Key words: Glass-ionomer cement; fluorides; ion release

# COMBINATION OF FULL- AND SPLIT-THICKNESS CORONALLY DISPLACED FLAP WITH CONNECTIVE TISSUE GRAFT IN THE GINGIVAL RECESSION TREATMENT

### Mirnić J.<sup>1</sup>, Đurić M.<sup>1,2</sup>, Gušić I.<sup>1,2</sup>, Veljović T.<sup>1</sup>, Vukoje K.<sup>1</sup>, Ramić B.<sup>1</sup>, Stojilković M.<sup>1</sup>

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#### Abstract:

Introduction:

Gingival recession often occurs in adults, tends to increase with age, and affects individuals with both low and high oral hygiene standard. The presence of recession is aesthetically unacceptable to most patients, dentine hypersensitivity may also occur, and exposed root surfaces are prone to the oral environment influence and may be associated with carious and non-carious cervical lesions. Aim: The aim of this paper was to present coronally displaced flap (combination of full and split thickness) with connective tissue graft as a root coverage procedure with minimal/no scar formation.

#### Case report:

A 22-year-old female patient with excellent oral hygiene presented to the Dentistry Clinic of Vojvodina due to gingival recession on tooth 24 (Müller class I) and sensitivity to cold. Apical to the recession, insufficient height and thickness of keratinized tissue was present, so coronally displaced flap in conjuction with connective tissue graft was performed.

#### Conclusion:

The clinical healing process was complete within 12 months. The root exposure was completely covered with newly formed keratinized tissue. The increase in attached gingival height and thickness with no scar formation was clinically evident, suggesting that the procedure has yielded the desired results.

Key words: gingival recession/surgery; coronally displaced flap; aesthetics

# IMPROVEMENT OF THE HYBRID LAYER MORPHOLOGY BY DENTINAL COLLAGEN CROSS-LINKERS

#### Pecikozić N.<sup>1</sup>, Lainović T.<sup>1</sup>, Maravić T.<sup>2</sup>, Novta E.<sup>1</sup>, Krmpot A.<sup>3</sup>, Blažić L.<sup>1,4</sup>, Rabasović M.<sup>3</sup>

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#### Abstract:

Contemporary research emphasizes tissue biomodification by additional cross-linking of collagen molecules to improve the quality and durability of the hybrid layer. Dentin modification by collagen cross-linkers improves its biochemical and biomechanical properties and makes it more resistant to enzymatic degradation1. Nonlinear laser scanning microscopy (NLSM) very efficiently images and analyzes biological substrates2.

This study aimed to analyze for the first time the morphology of the hybrid layer modified with collagen crosslinkers by means of NLSM.

The extracted teeth were prepared to the level of the middle dentin. After etching with 37% orthophosphoric acid for 15 seconds, rinsing, and drying, 0.3M EDC (1-ethyl-3(3dimethylaminopropyl)carbodiimide) was applied to dentin. The control group samples were prepared in the same way, but without EDC treatment. The samples from both groups were further bonded and restored with a resin composite. The prepared samples were cut into longitudinal slices and imaged by the NLSM at the Institute of Physics in Belgrade.

The NLSM images revealed the hybrid layer morphological changes caused by EDC. In both groups, dentinal tubules were open, as a result of acid etching. The images of the experimental group showed deeper and more evenly distributed resin tags, and a wider and more homogenous hybrid layer compared to the control group. Adhesion and cohesion fractures of the hybrid layer were more frequently present in the control group.

The hybrid layer modified by EDC is more homogenous, contains deeper and uniformly distributed resin tags, and it seems to be more resistant to adhesion and cohesion fractures.

Keywords: dentin, hybrid layer, collagen cross-linkers, EDC, NLSM

#### Acknowledgments

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# COST ACTION 21122- PROMOTING GERIATRIC MEDICINE IN COUNTRIES WHERE IT IS STILL EMERGING. (PROGRAMMING)

#### Popovac A.<sup>1</sup>, Puškar T.<sup>2</sup>, Jeremić Knezević M.<sup>3</sup>, Veselinović M.<sup>4</sup>, Sazdanić Velikić D.<sup>5</sup>, Stančić I.<sup>1</sup>

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#### Abstract:

#### **INTRODUCTION**

Geriatric Medicine (GM) is the field of medicine that is concerned with the health and well-being of older adults and can play a crucial role in the alignment of health systems to the needs of the constantly growing older populations. Countries have varying GM development backgrounds in Europe. Pragmatic solutions that aim to address the specialized health care needs of older people, such as tailored education and training of the existing workforce, may be feasible, affordable, exponentially efficient, and, thus, highly relevant.

#### **OBJECTIVES**

The PROmoting GeRiAtric Medicine in countries where it is still eMergING. (PROGRAMING, CA-21122) main objective is the definition of the content of targeted education and training activities in GM forhealth care professional across various clinical settings, destined mainly for countries where GM is still emerging and adapted to the local context, the needs and assets of stakeholders and the pragmatic possibilities of involved settings.

#### METHODS

PROGRAMING, CA-21122, is formed by 249 researchers from them 189 from inclusiveness countries target, 177female, 105 young researchers. 39 countries, and includes 5 Working Groups (WG) with the following objectives: 1) State-of-the-art mapping and needs' assessment, 2) Definition of the content of the training course in GM destined for professionals working in ambulatory settings and home care networks, 3) Definition of the content of the training course in GM destined for professionals working in acute/subacute and long term care settings, 4) Framework for training methods, and 5) Dissemination and impact maximization.

The participation of researchers from Serbia in this project is important, given that we are designated as a "target country", that is, an area where knowledge of geriatric medicine and dentistry needs to be improved, so that in the future we can adequately deal with the treatment and care of the growing number of old people. The research team of Serbia consists of scientists and clinicians from the Faculty of Dentistry, Medicine and Pharmacy in Belgrade as well as the Faculty of Medicine in Novi Sad. One of the goals of the action is certainly the exchange of knowledge with fellow experts in geriatric medicine from the developed countries of Europe, the expansion of research on an international and interdisciplinary level. Geriatric medicine and dentistry are closely related and cannot be separated when considering the well-being of the elderly. Given that systemic changes, as the ultimate goal, are not possible in a short time, the starting point is investment in the education system and training of young people.

#### EXPECTED RESULTS

To raise awareness and promote the benefits of the specific approach of GM to the health and wellbeing of older people among health care professionals, policymakers, and older people, PROGRAMMING, CA-21122, will highlight the current state of GM in Europe until 1/11/2026, and

provide a consensus on the content of brief targeted education and training activities in GM for health care professionals across various clinical settings and the creation of an international network of GM-related professionals and the ambition to engage stakeholders and influence policy makers for the sake of GM in countries where it is still emerging.

Key Words: Geriatric Medicine - Professional Education - Older people - Health care.

"The results presented in this paper were realized as a part of the project no. CA21122, titled "PROmoting GeRiAtric Medicine in countries where it is still eMergING. (PROGRAMMING)" funded by the European Union.

# THERAPY OF DISTAL BITE BY TWIN BLOCK APPLIANCE

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#### Abstract:

The twin block (TB) is a functional appliance used to correct the skeletal malloclusion during the growth phase. The indications for TB are Class II div 1, class II div 2, deep bite, dentoalveolar type of the open bite, cross bite, TMJ therapy, growth modification during mixed dentition, abnormal orofacial habits prevention, etc. The advantage of TB is easy adjustment, allows normal oral functions especially talk. Therapeutic effects of TB are skeletal, dentoalveolar and muscular equilibrium. Comfortable and easy to wear, suitable in controling the backward rotation of the mandible, and also can be used for the teeth treatment reposition.

Key words: backward rotation of the mandible; bimaxillary appliance; distal malocclusion.

# APPLICATION OF SPHERICAL TIO<sub>2</sub> NANOPARTICLES AS MODIFICATION OF GLASS-IONOMER CEMENTS

# Ramić B.<sup>1</sup>, Cvjetićanin M.<sup>1</sup>, Drobac M.<sup>1</sup>, Vukoje K.<sup>1</sup>, Kantardžić I.<sup>1</sup>, Veljović T.<sup>1</sup>, Mirnić J.<sup>1</sup>, Maletin A.<sup>1</sup>

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#### Abstract:

Introduction: Because of the greater surface-to-volume ratio and antibacterial properties of nanoparticles (NP), they have found applications in restorative dentistry. Among the various existing nanomaterials, titanium dioxide ( $TiO_2$ ) has been the subject of numerous studies as an inorganic additive due to its chemical stability, biocompatibility, and potential improvement in the mechanical properties of dental restorative materials.

Aim: To microscopically assess whether the  $TiO_2$  forms clusters and agglomerates when incorporated into the ionomer matrix.

Methods and Materials: Two commercially available GIC materials - Fuji IX (Fuji IX GP, GC International, Japan) and Ketac Molar (Ketac Molar EasyMixTM- 3 M/ESPE, Maple-wood, Minnesota, USA) were incorporated with  $TiO_2$  powder at 5% in the form of spheres NP with an average size of 20 nm. The flexural strength of both modified GIC materials was tested and the obtained fracture surface was analyzed using SEM.

Results: The microscopic images of both tested  $TiO_2$  modified GICs showed the formation of microscopic clusters of these NPs on the tested surface without microscopically visible signs of their uniform incorporation into the ionomer matrix.

Conclusion: When  $TiO_2$  nanoparticles are dispersed in a liquid, they are showing a high surface energy, which can be reduced by the formation of clusters. Understanding the factors that drive nanoparticle clustering is important for developing effective strategies to control their aggregation, which is critical for many nanotechnology applications. This study confirmed that spherical  $TiO_2$ -NPs are not recommended as a potential modifier of GIC materials because their uniform distribution throughout the mixed material is very difficult to achieve.

# KNOWLEDGE OF DENTISTS AND PHYSICIANS ABOUT THE ASSOCIATION BETWEEN PERIODONTITIS AND SYSTEMIC DISEASES

### Stojilković M.<sup>1</sup>, Gušić I.<sup>1,2</sup>, Dejanović K.<sup>3</sup>, Pecikozić N.<sup>1</sup>, Veljović T.<sup>1</sup>, Mirnić J.<sup>1</sup>, Đurić M.<sup>1,2</sup>

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#### Abstract:

There is a strong body of evidence that supports the association between periodontitis and certain systemic diseases and conditions. The aim of the present study was to assess the knowledge of physicians and dentists in Serbia with regard to the relationship between periodontal and systemic health. The self-reported questionnaire was sent to 3000 e-mail addresses of randomly selected dentists and physicians who work at government and private practice in different cities in Serbia. The survey was consisted of questions about the demographic characteristics of participants, as well as their knowledge about the relationship between periodontitis and systemic diseases. The questionnaire was completed by 1301 health care providers, 739 (57%) physicians and 562 (43%) dentists. Most participants (95%) agreed that there was a relationship between periodontitis and general health. Knowledge about this association was significantly higher among dentist, compared to physicians  $(\gamma 2=50.913, p<0.001)$ . Diabetes mellitus was the most frequent systemic disease known to be related to periodontitis (70% physicians, 86% dentists). Only 40% of physicians and 59% of dentists were aware of the association between periodontitis and ischemic heart disease. The smallest number of participants recognized periodontal disease as a possible risk factor for premature birth (34% physicians, 46% dentists). Although the most of participants were aware of the connection between periodontitis and systemic diseases, the results show that this was not based on specific knowledge. This indicates the need for further education of physicians and dentists in this area.

Key words: periodontitis; non-communicable diseases; parodontal medicine

# LIP PRINT PATTERN AS AN IDENTIFICATION TOOL

# <u>Veljović T.<sup>1</sup></u>, Veselinović I.<sup>1,2</sup>, Đurić M.<sup>1,3</sup>, Gušić I.<sup>1,3</sup>, Mirnić J.<sup>1</sup>, Maletin A.<sup>1</sup>, Ramić B.<sup>1</sup>, Vukoje K.<sup>1</sup>

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#### Abstract:

Lip prints are considered an important form of evidence transfer, analogous to fingerprints, as the arrangement of lines on the red part of human lips is unique to each individual and can thus be very useful in forensic investigations and personal identification. Not only are lip prints uniform throughout one's life, but the available evidence shows that, after lip trauma, the lip pattern eventually reverts to that prior to the injury. Although lip prints are commonly found on drinking glasses, cutlery, paper napkins, cigarette butts, and tissues, they can be retrieved from the surface of windows, paintings, doors and plastic bags, making them a useful tool for establishing a link between the subject and the crime scene. In some cases, they can appear on food products alongside bite marks. These prints are a result of humidity present in lips through saliva, mixed with the oils secreted by neighboring salivary and sebaceous glands. These substances, particularly the lipids and fatty acids, are transferred to an object through lip contact which can be rendered visible through different forensic techniques.

The most commonly used classification for recording lip patterns was established by Suzuki and Tsuchihashi by studying the natural lip marks or fissures, resulting in the following six types: Type I - Complete vertical; Type I' - Incomplete vertical; Type II - Branched grooves (Y-shaped pattern); Type III - Intersected grooves; Type IV - Reticular pattern; and Type V - Irregular.

Key words: lip prints, forensic anthropology, personal identification

# **BIOLOGICALLY BASED THERAPIES IN ENDODONTICS - LATEST STANDPOINTS FOR VITAL PULP THERAPY**

# <u>Vukoje K.<sup>1</sup></u>, Ramić B.<sup>1</sup>, Kantardžić I.<sup>1</sup>, Cvjetićanin M.<sup>1</sup>, Stojšin I.<sup>1,2</sup>, Mirnić J.<sup>1</sup>, Veljović T.<sup>1</sup>, Maletin A.<sup>1</sup>

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#### Abstract:

Introduction: Vital pulp therapy (VPT) techniques for years were means for preservation of the radicular pulp in immature adult teeth. Today, according to the latest position statement of the European Society of Endodontology (ESE) from 2019 and American Association of Endodontists (AAE) from 2021, the focus of VPT is broader; including teeth previously thought to have irreversibly inflamed pulps.

Rationale: Maintaining the health and vitality of the pulp, preventing apical periodontitis and developing minimally invasive biologically based therapies became important themes within contemporary clinical endodontics. The current AAE diagnostic terminology assigns a vital pulp to one of three categories: normal pulp, reversible or irreversible pulpitis (which could be symptomatic or asymptomatic). Based on clinical, biological and theoretical considerations, there is no clear boundary for the irreversibility of the pulpal disease and according to this even symptomatic pulps may be candidates for VPT. More precise gradation suggests the following terminology: initial, mild, moderate and severe pulpitis. This standpoint strongly advise the complete removal of demineralized infected dentin, the use of magnification (especially if the pulp becomes exposed) and the use of biomaterials such as calcium silicate cements in VPT procedures due to their immunomodulatory effects.

Conclusion: There is a need to promote minimally invasive treatment strategies in Operative Dentistry and Endodontology; however, the development of accurate diagnostic tools, evidence-based protocols and education in management of the exposed pulp are critical in the future. The presented position statement also encourages additional clinical trials to assess long-term outcomes of such VPT and a review of the endodontic diagnostic terminology.

Key words: deep caries management, direct pulp capping, endodontics, pulpotomy, vital pulp therapy

# ORAL MUCOSAL LESIONS IN CHILDREN

# <u>Horvat Aleksijević L.<sup>1</sup></u>, Tomas M.<sup>1</sup>, Petrović D.<sup>1</sup>, Juzbašić M.<sup>1</sup>, Dragičević D.<sup>1</sup>, Pul L.<sup>1</sup>, Lešić N.<sup>1</sup>, Seifert D.<sup>1</sup>

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#### Abstract:

Childhood diseases are a continuous source of interest in dental medicine. Congenital, developmental, and hereditary diseases may either be present upon birth or appear in early childhood. Developmental anomalies can indicate systemic disorders. Childhood age frequently brings about benign tumors and different types of traumatic lesions to the oral mucosa. Recurrent aphthous lesions are the most common type of ulcerations in childhood, and their etiology is considered multifactorial. Oral mucosal lesions in children require different treatment approaches depending on etiological factors and clinical presentation. Clinicians should have adequate knowledge of oral anatomy in order to diagnose and treat pathological conditions.

Key words: children; oral mucosa; pediatric oral lesions

# THERAPY PLANNING FOR SUCOMPLETLY EDENTULOUS PATIENTS

### Puškar M.<sup>1</sup>, Puškar N.<sup>1</sup>, Jeremic-Knezevic M.<sup>1</sup>, Djurovic-Koprivica D.<sup>1</sup>, Maletin A.<sup>1</sup>

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#### Abstract:

Therapy plan for subcompletly edentulous patient is challenging phase in prosthetic treatment. If the patient has one, two or three teeth remaining in the lower jaw, the aim is to use them for retention and stabilization improvement. In the upper jaw the situation can be considered on different way, because there is many times greater surface of the bearing tissues for loading acceptance, retention and stabilization than in the lower jaw. The thorough evaluation of the periodontal status, clinical crown to clinical root ratio and hard dental tissue should be done. After analyzing of OPT radiogram, study models should be cast in the articulator after interjaw relationship determination. It would give us the information if the remaining teeth are disturbing occlusal plane, if shortening of the crowns is necessary etc. Shortening the crowns of the remaining teeth will improve clinical crown-clinical root ratio. Overdenture is good therapy option in the lower jaw if there is enough space between the remaining teeth and upper residual alveolar ridge. If there is not enough intermaxillar space and if financial limitations are present, partial acrylic denture can be made. If the teeth should be covered with crowns, it is important to determine final interjaw relationship while making fixed restoration, although there is n remaining teeth in the upper jaw. Complexity of the treatment plan in subcompletly edentulous patient can be illustrated by case reports that were treated on the Clinic of Dentistry of Vojvodina.

Key words: partial denture, subcomplete edentulism, therapy plan

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# TESTS FOR INVESTIGATION OF CYTOTOXICITY IN DENTISTRY

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#### Abstract:

Cytotoxicity testing is one of the first steps in testing biocompatibility of the materials. The toxicity of materials can be evaluated using in vitro tests, tests on experimental animals and clinical trials. In vitro cytotoxicity studies indicate basic cellular toxicity by analyzing different parameters: by measuring cell viability, cell proliferation, cell membrane preservation, by measuring DNA synthesis or monitoring cell metabolism. Dye exclusion test with trypan blue for evaluation of cell membrane integrity, i.e. cell viability, agar diffusion test, that assess integrity of cell membranes ie. cell lysis, tests with tetrazolium salts that assess mitochondrial function among which is the MTT test are widely used for cytotoxicity testing. In contemporary research, possible harmful effects of dental materials are determined using a large number of in vitro and in vivo tests. The advantage of the tests on cell cultures is that each agent and its influence on cell growth and replication can be tested. In vitro research always precedes clinical research, directs it and shortens the time required to reach the result.

When using new substances clinical research starts after obtaining the results of in vitro trials and gaining results on experimental animals that substances are safe enough to be used. The paper presents various tests for cytotoxicity examination used by the research team to test dental materials. For the evaluation of cytotoxicity, it is necessary to use several different in vitro tests, that examine different cell functions, because using only one method does not provide enough information about the examined dental material that would lead to a valid conclusion.

Key words: cytotoxicity, dental materials, cell lines

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# FREQUENCY OF RUBBER DAM USE AMONG DENTIST IN SERBIA AND REGION

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#### Abstract:

Introduction: Rubber dam is an aid used in dentistry to isolate teeth from other parts of the oral cavity during various dental interventions. Although it is often recommended due to its advantages, its use is often neglected in clinical practice.

Aim: The aim of this study was to assess the frequency of use of rubber dam during dental procedures in Serbia and the region, as well as collecting experiential views and opinions of dentists or specialists on the use of rubber dam, which would further determine the influence factors which encourage or discourage its use.

Material and Methods: Data were collected by surveying the target group using an originally created online questionnaire, which was distributed through social networks and through acquaintances within the target group.

Results: Based on the results of this study, 39.53% of dentists are using rubber dam, of which only 6.31% always use a rubber dam. The majority of respondents (60.47%) do not use a rubber dam. The use of rubber dam is more common in private practice and among male dentists. The vast majority of dentists who use rubber dam opt for its use during endodontic treatment and less during restorative procedures.

Conclusion: The use of rubber dam is more often neglected in restorative dentistry than in endodontics. Gender, years of experience as well as the type of practice (public or private) are factors that affect the use of rubber dam during dental procedures.

Key words: rubber dam; endodontics; restorative dentistry.